

Antimicrobial resistance.

I. Antibiotic history and development

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■ INTRODUCTION

Since humans appeared on earth they have evolved together with microorganisms that existed for a long time before. These creatures, despite being the smallest, have caused the worst diseases that decimated human populations since there is any record.

This year marks the 90th anniversary of the discovery of penicillin in 1928 by Alexander Fleming, considered the beginning of the “modern antibiotic era” in medicine. Definitely a turning point in the treatment of infectious diseases, antibiotics have saved many millions of lives. Together with vaccination they entirely changed the approach and the morbidity and mortality of most infections. Unfortunately systematic overuse and misuse may compromise their effectiveness, as rapidly rising antibiotic resistance is causing the appearance of multidrug resistant microorganisms. At the same time an alarming decline in the production of new effective antibiotics is observed. Many, WHO among them, are already predicting the coming of a “post-antibiotic era.”

To fully comprehend the success and failure of antibiotics and the emerging resistance to them, an understanding of their history, origins, evolution and functions is indispensable, as well as the main mechanisms for gene transfer in microorganisms.

■ TRANSMISSION OF BACTERIAL GENES

Vertical transmission (Figure 1) in which a parental cell divides in two, passing the entire genome to the progeny, depending for adaptation solely on mutational changes in the genome and their vertical gene transfer, a very slow evolutionary process. Therefore, the evolutionary history of bacterial genes, especially those involved in the biosynthesis of antibiotics and other secondary metabolites, depends to a great extent on the more rapid and widespread horizontal gene transfer (Figure 2).(1,2)

Horizontal transmission of genetic information includes three different processes. *Conjugation*, considered the bacterial equivalent of sexual reproduction or mating, involves the exchange of genetic material by direct cell-to-cell contact. The success of the gene transfer often depends on its benefit to the recipient, which may include antibiotic

resistance, improved adaptation to the environment or the ability to use new metabolites.

Transformation results from the uptake of DNA from the environment.

Transduction is the process in which genes from a bacterial host cell become integrated into the genome of a bacterial virus (bacteriophage); part of the infected cell's DNA is packed by mistake into the capsid of newly formed virions and carried to another host cell when the bacteriophage initiates another cycle of infection.(1)

■ PREHISTORY AND HISTORY OF ANTIBIOTICS

Ancient civilizations were exposed to antibiotics. Traces of tetracycline, were found in human bones from ancient Sudanese Nubia dating back to 350–550 AD.(3) The distribution of tetracycline in bones is only explicable by a diet, which consisted of wheat, barley and millet. Contamination of stored grains provided the proper environment for tetracycline-producing *Streptomyces*. Its ingestion, at apparently therapeutic levels, may explain the low level of infectious disease found amongst these people.

Another example of ancient antibiotic exposure was found in bone and teeth samples from the late Roman period in the Dakhleh Oasis, Egypt. This is consistent with the presence of tetracycline in the diet. Comparisons with tetracycline labeling in patients under treatment with this drug resulted in very similar labeling. Lack of signs of bone infection in these samples suggests a protective effect of the ingested antibiotic.(4)

Tetracyclines are unique among antibiotics in that they are incorporated into the hydroxyapatite mineral portion of bones as well as tooth enamel, providing permanent markers. Exposure to other antibiotics in ancient populations can only be detected by surviving cultural or anecdotal evidence. An example is the historical use of the red soils in Jordan, still in use today, for skin infections. *Actinomycete* bacteria, producing actinomycin C2 and actinomycin C3, were isolated from these soils.(5)

There are many references from ancient Egypt, China, Serbia, Greece and Rome to the beneficial effect of the topical application of moldy bread on wounds. In the Jewish Talmud, the therapeutic use of kutach b'avli or chamka, a mash of moldy corn soaked in water or date wine is mentioned. (6) John Parkinson (1567–1640), London apothecary and King's herbarian, refers to the curative effect of molds in his book *Theatrum Botanicum*, published in 1640.(7,8)

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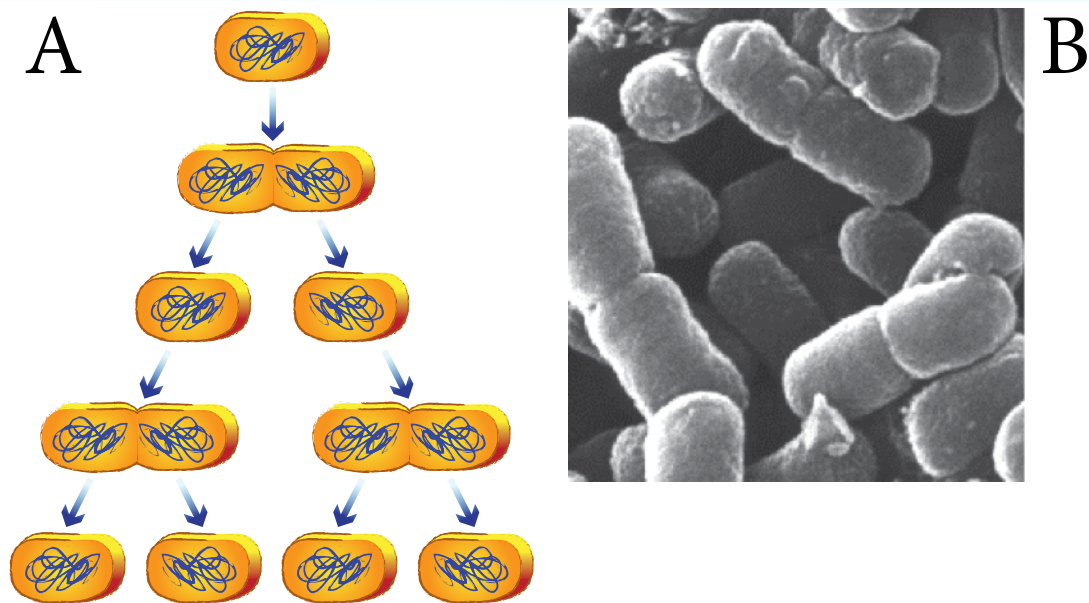


Figure 1 Vertical transmission of genetic information

A) Diagram of two replication cycles B) Electron micrograph of dividing *E. coli* cells

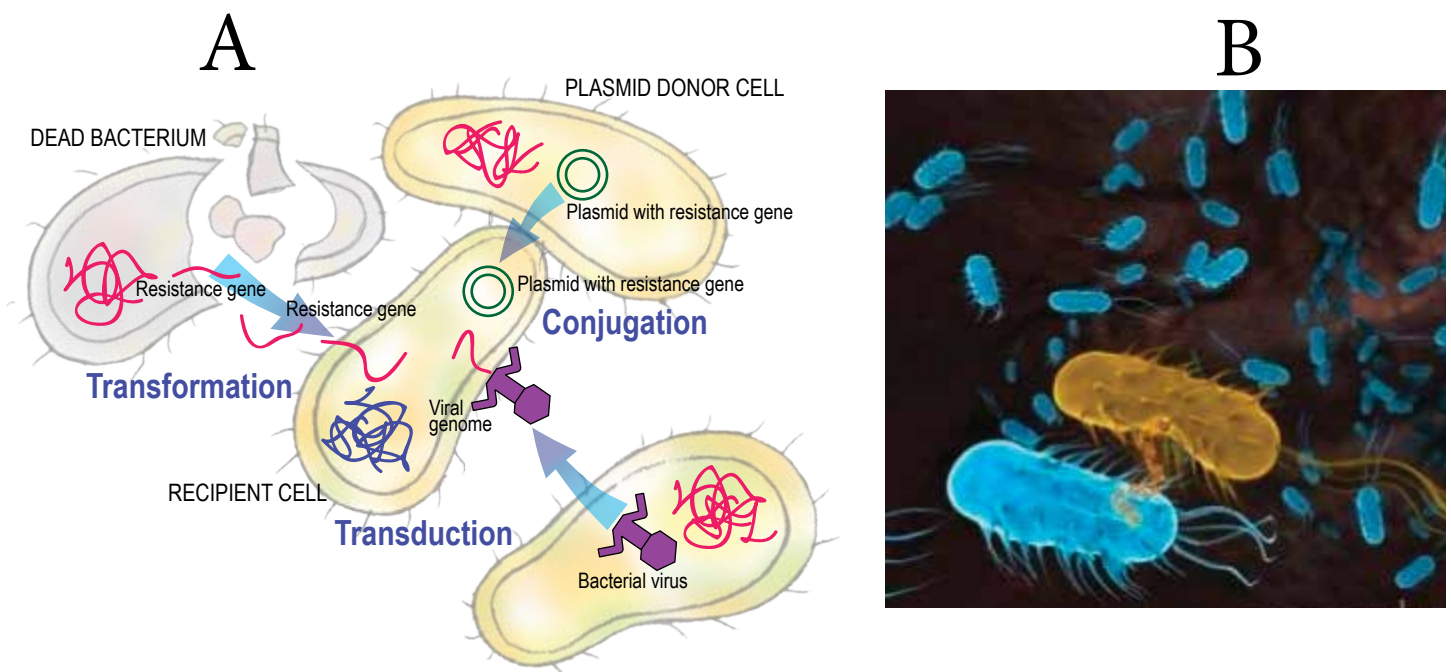


Figure 2 Horizontal transfer of genetic material between two unrelated cells.

A) diagram of conjugation, transformation and transduction; B) Electron micrograph showing horizontal gene transfer.

Remedies used in traditional medicine are another possibility of exposure to antimicrobials in the pre-antibiotic era. The discovery in the 1970's of a potent anti-malarial drug, artemisinin, from extracts of *Artemisia* plants used as a remedy by Chinese herbalists for thousands of years is a good example.(5).

"New ideas" generally have precedents, people who envisioned some of the ideas we hold true today, but were unable to demonstrate them, due to lack of the necessary scientific and technological tools. The discovery

of microorganisms by Robert Hooke and Anthony Leeuwenhoek in the 17th century and the validation of the germ theory by Louis Pasteur in the 19th century, prompted a long search for substances that could serve as "magic bullets"*(9). And in his famous postulates, Robert Koch indicated that specific bacteria were responsible for specific diseases. These advances set the stage for a rational search for substances that would kill microorganisms that cause

* Paul Ehrlich's expression used for specific substances which have affinity for pathogenic organisms at which they were aimed, killing them, without harming the host.

infectious diseases.

Jean Paul Vuillemin introduced the term “antibiosis”, meaning “against life” in his 1889 paper “Symbiose et antibiose” to describe the struggle of some microorganisms against competing ones. Antibiosis had already been described in 1877 in bacteria when Louis Pasteur observed that an airborne mold could inhibit the growth of *Bacillus anthracis*.⁽¹⁰⁾

While in 1945, Selman Waksman, who deceitfully received alone all the credit and the Nobel Prize of Medicine in 1952 for the discovery of streptomycin in a soil *Actinomycete*,⁽¹¹⁾ coined the term “antibiotic” for substances that kill bacteria or stop them from growing by interrupting some of their essential metabolic processes, but are mostly innocuous to human and animal cells. (2) These substances are produced by microorganisms as a defensive mechanism to destroy competing microorganisms.

Bartolomeo Gosio, an Italian medical scientist, discovered the effect of what he called mycophenolic acid in 1893. Its toxicity did not allow human use, but today derivatives are used for many different purposes, among them, as immunosuppressants in kidney, heart, and liver transplantation.^(12,13)

After unsuccessful research, Ehrlich found, in 1909, that arsenic compound number 606 had rapidly cured a rabbit with syphilis, showing no side-effects. After tests on some human patients, an unprecedented clinical trial with 65 000 doses was carried out. Only then was Salvarsan (its commercial name) to become the first antimicrobial marketed, it was not an antibiotic in the strict sense of the word.⁽⁵⁾ A decade later the incidence of syphilis in many countries was reduced by 75% or more. And patients with neurosyphilis had almost disappeared. Ehrlich was awarded the Nobel Prize for Medicine in 1908.⁽¹⁵⁾

In 1935, a team led by physician/researcher Gerhard Domagk discovered and developed the first sulfonamide at the Bayer Laboratories of the I.G. Farben company in Germany, a synthetic red dye more popularly known by its trade name of Prontosil. Prontosil was the first medicine to effectively treat a range of bacterial infections, resulting in a sharp decline in mortality due to diseases such as meningitis, post-partum infections and pneumonia. Domagk’s discovery saved many lives, including those of his own daughter, Winston Churchill and Franklin D. Roosevelt, Jr., son of then US President Roosevelt.⁽¹⁵⁾ It had no effect at all in the test tube, only in live animals. Later, it was discovered by Bovet and coworkers at the Pasteur Institute, that inside the body, the drug was metabolized into two parts, releasing a smaller, colorless, active compound called sulfanilamide. The discovery helped establish the concept of “bioactivation” and ended the German corporation's possibility of enormous profit, since the active molecule sulfanilamide (or sulfa), first synthesized in 1906, was widely used in the dye-making industry. Its patent had since expired and the drug was

available to anyone.

At the beginning of World War II, since there was no other antibiotic available, overuse of sulfa drugs caused the appearance of resistant microorganisms and allergies.

Domagk, awarded the 1939 Nobel Prize for Medicine, was forced by the Nazi government to decline. Hitler had banned acceptance of Nobel Prizes after Carl von Ossietzky, an anti-fascist, was awarded a Nobel Peace Prize. In 1947, in a formal award ceremony Domagk was presented with his Nobel Prize.⁽¹⁷⁾

■ THE ANTIBIOTIC ERA, A BRIEF HISTORY OF PENICILLIN

In 1923, Alexander Fleming, a Scottish bacteriologist, had already discovered lysozyme, an enzyme with strong bactericidal properties obtained from nasal fluid of a person with a “common cold”. Before that, he had warned in his Hunterian Lecture to the Royal College of Surgeons (1919) of the scarce and sometimes even harmful effect of antiseptics on wounds. This work was carried out during World War I in France in a laboratory under the direction of Dr. Almroth Wright. They concluded that antiseptics were not effective in deep wounds, because they did not reach the microbes and at the same time inhibited the action of leukocytes in the blood and pus, which are a natural and effective defense mechanism. He also went on to demonstrate that surprisingly some concentrations of the most used antiseptics even increased microbial growth.⁽¹⁷⁾

In 1928 Fleming, carried out a serendipitous discovery. Although, as Louis Pasteur said in his 1854 speech: “In the field of observation, chance favors only the prepared mind”. After checking his culture plates during several days, he found a contaminating mold in a culture of *staphylococci* that was encircled by a halo where bacteria seemed to have disappeared (Figure 3). He found the mold culture prevented growth of *staphylococci*, even when diluted 800 times and named the substance penicillin, because it was obtained from *Penicillium notatum*. Fleming’s research showed that the mold produced a substance that inhibited *staphylococci* and many other bacteria, as well (Figure 4). In his seminal work (On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*, 1929), Fleming studied the properties of the substance, the sensitivity or resistance of different microorganisms to it, its toxicity to animals.⁽¹⁸⁾ Crude filtrates of *Penicillium* cultures were used by Fleming in 1932 in patients. Difficulties in producing, stabilizing and purifying penicillin to obtain the required amounts for clinical testing made him abandon research on penicillin in the late 30’s. Fleming had tried in vain to get chemists interested in resolving persisting problems with purification and stability of the active substance and supplied the *Penicillium* strain to anyone requesting it.

Fortunately, an Oxford team led by Howard Florey and Ernest Chain produced sufficient amounts of crude extracts

to attempt clinical testing. Around August 1942, Florey and Chain sent some small amounts of crude penicillin extract to Robert Pulvertaft in the Central Pathological Laboratory in Cairo's General Hospital. Pulvertaft showed the "important role that it (penicillin) might have in the control of infections of war wounds" and that "sepsis [acute blood poisoning] as we know it might almost disappear if sufficient penicillin were available."⁽¹⁹⁾ At that time penicillin was produced in very small amounts. Many laboratories locally produced crude extracts, which due to differences among them gave varied results. Since penicillin was so quickly eliminated through the kidneys, it was also purified from urine and used for reinjection.⁽²⁰⁾

Fleming's screening method using inhibition zones in lawns of pathogenic bacteria on the surface of agar-medium plates required much less resources than testing in animal disease models and thus became widely used in mass screenings by many researchers in academia and industry. Fleming was also among the first who cautioned about the potential resistance to penicillin when used too little or for a too short period during treatment in his 1945 address in the Nobel Prize award ceremony.

As mentioned above, during World War I, there were only antiseptics to treat the soldiers' wounds with contradictory results. Many soldiers died from infected wounds.

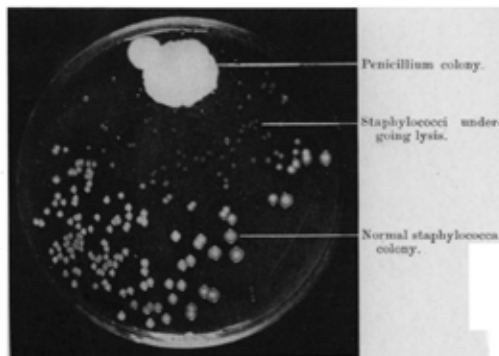


FIG. 1.—Photograph of a culture-plate showing the dissolution of staphylococcal colonies in the neighbourhood of a penicillium colony.

Figure 3. Copied from A. Fleming's original paper: *On the antibacterial action of cultures of a Penicillium, with special reference to their use in the isolation of B. influenzae*. *British Journal of Experimental Pathology*, 1929;10:222–36.

Many consider that, to a certain extent, World War II was won thanks to the availability of penicillin that substantially reduced morbidity and mortality due to infections. In 1945 Alexander Fleming, Howard Florey and Ernest Chain were jointly awarded the Nobel Prize in Medicine.

It is not possible to overstate the importance of the work to eradicate, prevent or control the scourge of infections that had afflicted humanity forever. The great number of Nobel Prizes awarded to researchers who advanced the frontiers of knowledge on infections and antibiotic therapy is the best

demonstration.

The global antibiotic market is one of the largest in the pharmaceutical industry. It is predicted to reach 57 billion (US) by 2024.⁽²¹⁾

■ ANTIBIOTICS: MECHANISMS OF ACTION, CLASSIFICATION AND USE

For clinical use bacteria are divided into broad categories, according to whether they retain the violet dye of the Gram test or not. The two groups have different features, which make them sensitive to some antibiotics, while resistant to others. It is also a fast method for differentiating bacteria species.

Antibiotics are a part of the antimicrobials, a larger group which also includes anti-virals, anti-fungals, and anti-parasitic drugs. Today most antibiotics are semi-synthetic or synthetic. Antibiotics can be grouped in many ways. One is by their mode of action. They are *bactericidal*, when they kill the target bacteria. They are *bacteriostatic* when they stop bacterial spread by interrupting their growth or replication without killing them. Their toxicity is selective, because they do not harm human or animal cells. They are also grouped according to their possibility of being effective against many infectious agents: *broad-spectrum* or to only one or a few: *narrow-spectrum*.

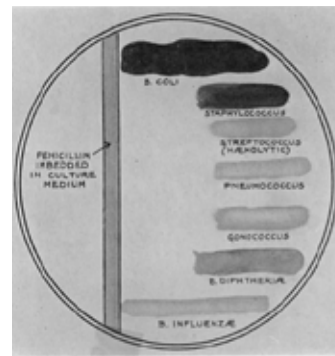


FIG. 2.

Figure 4. Copied from A. Fleming's original paper: *idem*

They are also classified by their type and use. The following are the main groups.^(20,22,24) See Table 1 below for an overview.

BETA-LACTAM ANTIBIOTICS

This group includes penicillins and cephalosporins.

Penicillins

Penicillins, the oldest class of antibiotics work by inhibiting peptidoglycan cross-linkage. Modifications have extended their antibacterial spectrum and improved absorption.

They are used to treat skin infections, dental infections, ear infections, respiratory tract infections, urinary tract infections, and gonorrhea. Penicillins now include: natural penicillins (e.g. benzylpenicillin, penicillin V); penicillinase-resistant penicillin (e.g. flucloxacillin); aminopenicillins (e.g. ampicillin-like agents); extended-spectrum penicillins (e.g. piperacillin); penicillins combined with β -lactamase inhibitors (e.g. amoxicillin and clavulanate, known as co-

amoxiclav).

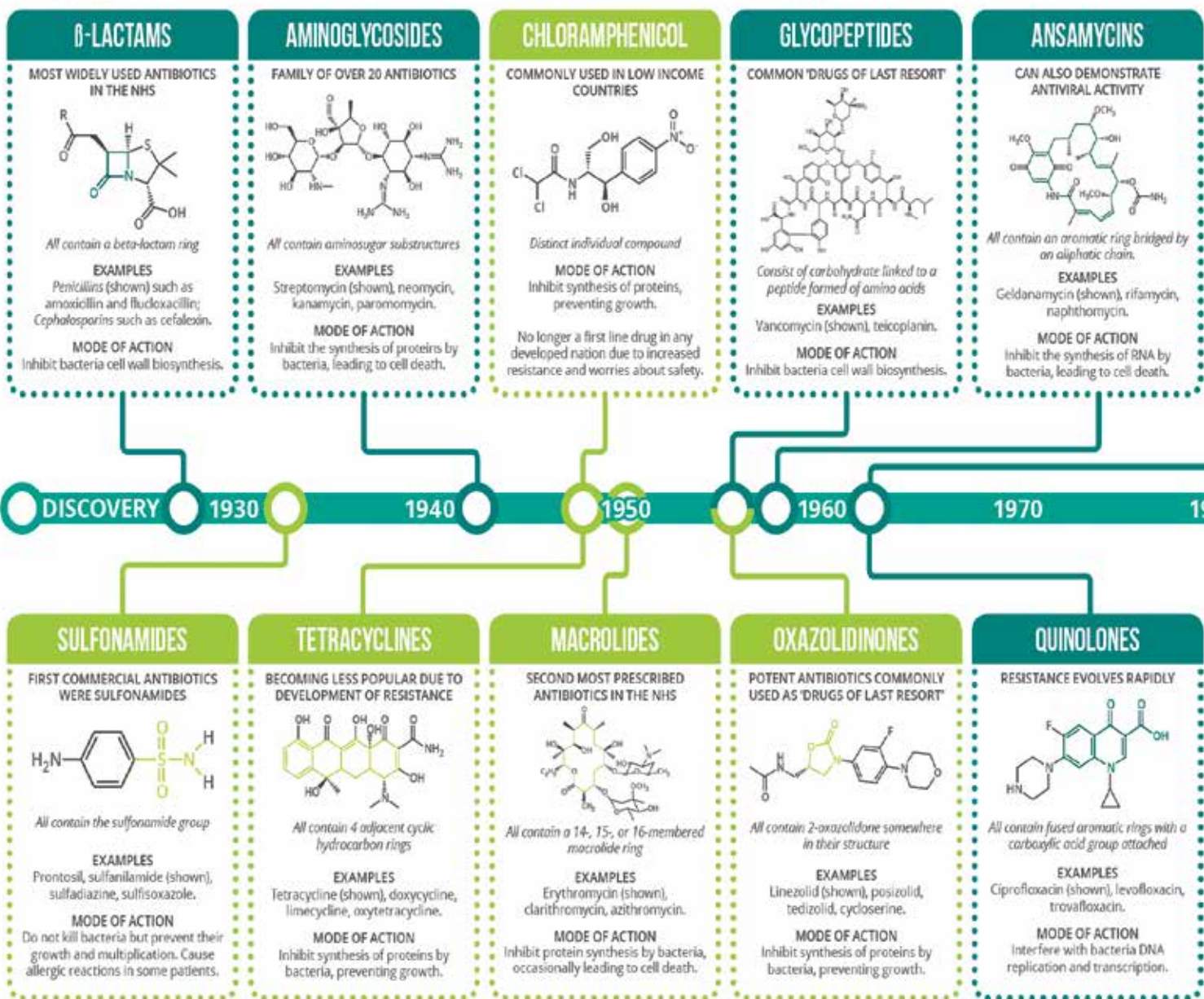
Oral absorption varies: benzylpenicillin (penicillin G) is unstable in the presence of gastric acid and must be given intravenously, but penicillin V is stable and can be given orally. The aminopenicillins and flucloxacillin are also administered orally, while the remaining agents must be given intravenously. Penicillins are excreted by the kidney and have a short half-life. They do not cross the blood-brain

DIFFERENT CLASSES OF ANTIBIOTICS - AN OVERVIEW

Key:

COMMONLY ACT AS BACTERIOSTATIC AGENTS, RESTRICTING GROWTH & REPRODUCTION

COMMONLY ACT AS BACTERICIDAL AGENTS, CAUSING CELL DEATH



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barrier unless the meninges are inflamed.

Cephalosporins

Cephalosporins are closely related to penicillins. They are derived from cephalosporin C which is produced from *Cephalosporium acremonium*. They show activity against Gram-positive organisms and later compounds have activity against Gram-negative bacteria including *Pseudomonas*.

They cause very few side-effects. Some cephalosporins cause thrombocytopenia, neutropenia, abnormalities of platelet function and coagulation. Approximately 5–10% of patients with allergic hypersensitivity to penicillins will also have cross-reactivity with cephalosporins.

They are used to treat pneumonia, strep-throat, tonsillitis, staphylococcal infections, bronchitis, otitis media, various types of skin infections, gonorrhea, urinary tract infections. Cephalosporin antibiotics are also commonly used for surgical prophylaxis. Cephalosporins are grouped into “generations”, where the newer is more effective than previous ones.

AMINOGLYCOSIDES

Aminoglycosides act by preventing translation of mRNA into proteins. They are administered parenterally, limited to the extracellular fluid and excreted in the urine. Aminoglycosides are toxic to the kidney at amounts close to therapeutic levels, which necessitates careful monitoring of serum concentrations. They also show high ototoxicity.

GLYCOPEPTIDES

The glycopeptides (vancomycin and teicoplanin) inhibit peptidoglycan cross-linking in Gram-positive organisms only. Resistance to them is rare, but sometimes found in enterococci (glycopeptide-resistant enterococci – GRE) and in some *Staphylococcus aureus*. They are not absorbed orally, thus are administered intravenously or intraperitoneally, except the oral use of vancomycin to treat pseudomembranous colitis. They are distributed in the extracellular

fluid, but do not cross the blood–brain barrier unless there is meningeal inflammation. They are excreted through the kidneys.

QUINOLONES

Quinolones act by inhibiting bacterial DNA gyrase. Early quinolones did not reach high levels in the tissues and were used only for urinary tract infections.

Fluorine modification (fluoroquinolones) are the newest class of antibiotics. Their generic name often contains the root “floxacin”. They are synthetic compounds, and are not derived from bacteria. They are active against Gram-negative pathogens including *Chlamydia*. They are generally well tolerated and have acceptable level of safety. The most common side effects, as for most antibiotics, include nausea, vomiting, diarrhea, abdominal pain. Quinolones are well absorbed orally, are widely distributed and penetrate cells well.

Newer products (e.g. moxifloxacin) are active against Gram-positive pathogens, including *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*.

MACROLIDES

The macrolides (erythromycin, azithromycin and clarithromycin) bind to the 50S ribosome, preventing protein synthesis; they are active against Gram-positive cocci, many anaerobes (but not *Bacteroides*), *Mycoplasma* and *Chlamydia*. They are absorbed orally, distributed in the total body water, cross the placenta, are concentrated in macrophages, polymorphs and the liver and excreted in the bile. Erythromycin, the prototype of this class, has a spectrum and use similar to penicillin. It may cause nausea. The newer macrolides (e.g. azithromycin) have more favorable pharmacokinetic and toxicity profiles. They are derived from *Streptomyces* bacteria.

OXAZOLIDINONES

The oxazolidinones (e.g. linezolid) inhibit protein synthesis at the 50S ribosomal subunit. They are most active against Gram-positive bacteria and are used mainly for the treatment of resistant Gram-positive infections. Linezolid is well absorbed orally and concentrated in the skin.

TETRACYCLINES

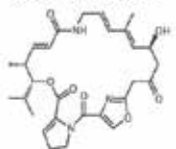
Tetracyclines act by inhibition of protein synthesis. They are active against many Gram-positive and some Gram-negative pathogens, *Chlamydia*, *Mycoplasma*, *Rickettsia* and *Treponemes*, *Plasmodium* and *Entamoeba histolytica*. Doxycycline is absorbed orally, has a long half-life and is widely distributed; adequate therapeutic levels may be obtained by a once-daily dosage. Tetracyclines should not be given to children under 8 years because they may cause gray to yellow discoloration of actively forming teeth and deposition in growing bones. They rarely cause allergies; side-effects include stomach cramps, diarrhea, nausea, vomiting, esophageal ulceration, sore mouth or tongue. The newer tetracyclines such as tigecycline are used to treat multiresistant Gram-negative infections.

OVERVIEW

NG BACTERIAL CELL DEATH

STREPTOGRAMINS

TWO GROUPS OF ANTIBIOTICS THAT ACT SYNERGISTICALLY



Combination of two structurally differing compounds, from groups denoted A & B

EXAMPLES

Pristinamycin 11A (shown),
Pristinamycin 1A.

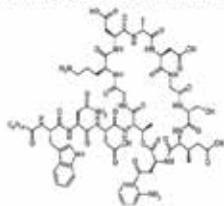
MODE OF ACTION

Inhibit the synthesis of proteins by bacteria, leading to cell death.

980

LIPOPEPTIDES

INSTANCES OF RESISTANCE RARE



All contain a lipid bonded to a peptide

EXAMPLES

Daptomycin (shown), surfactin.

MODE OF ACTION

Disrupt multiple cell membrane functions, leading to cell death.



SULPHONAMIDES AND TRIMETHOPRIM

Sulphonamides and trimethoprim act by inhibiting the synthesis of tetrahydrofolate. They are now rarely used in the treatment of bacterial infections but have an important role in the management of *Pneumocystis jiroveci* and protozoan infections including malaria. Sulphonamides can be given intravenously and are well absorbed when given orally. They are widely distributed in the tissues and cross the blood-brain barrier. They are metabolized in the liver and excreted via the kidney.

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