Introduction 2

Many of the organisms living around, on, and in human beings are too small to be seen without a microscope. They include viruses, bacteria, fungi, and protozoa (figure 2-1).

Viruses are short lengths of genetic material—deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—enclosed in a protein coat. So small that they have no room for the structures and processes for the biochemistry of their replication, viruses are obligate internal parasites. They must invade cells—human, animal, plant, or bacterial, depending on the virus—take over the cells' genetic apparatus, and direct the biochemistry of the cell to produce viral nucleic acid and protein and package them into new viruses.

Bacteria, the single-celled organisms that are the subject of this report, carry the structures and functions necessary for their replication in their cytoplasm. They generally are about one thousandth of a millimeter wide and nearly 500 times smaller than the average animal cell (Watson et al., 1986.). Bacteria are classified as **prokaryotes** because, unlike **eukaryotes,** such as fungi, protozoa, plants and animals, they have no internal membrane (the nuclear envelope) separating their genetic material from other components of the cell (figure 2-2). Bacteria differ from eukaryotes in having some molecular structures and biochemical processes that are absent from eukaryotes or that differ in significant ways from those of eukaryotes. Most antibiotics[1] work by interfering with a structure or process that is present in bacterial and not in other cells. This selectivity accounts for the rarity of serious side-effects associated with most antibiotics; the drugs find no good targets in human (or other eukaryotic cells) and cause few effects there. Figure 2-3 illustrates the differential effects of penicillin on animal cells, which do not have cell walls, and bacteria, which do, and a photo shows the destruction of a bacterial cell by penicillin. **Antibiotics have no effect on viral infections**; viruses use the molecular structures and functions of the infected cells and viral-infected cells offer no targets for antibiotics.

Fungi and **protozoa** are eukaryotes. Antibiotics have no effect on most of these microorgan-

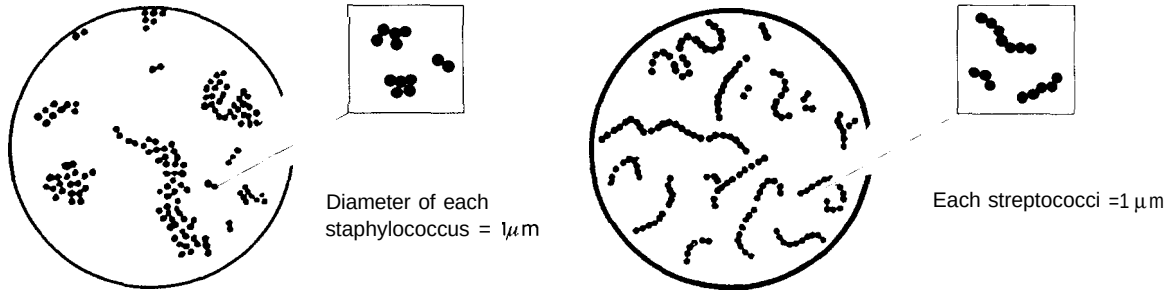
[1] OTA uses the term "antibiotics" to refer to substances that kill or inhibit the growth of bacteria. It is sometimes used to refer to substances that kill or inhibit organisms other than bacteria, but it is used here only to refer to substances with antibacterial activity.

FIGURE 2-1: Infectious Microbial Agents

VIRUSES

Adenovirus (colds) Retrovirus (AIDS) Herpes (fever blisters)

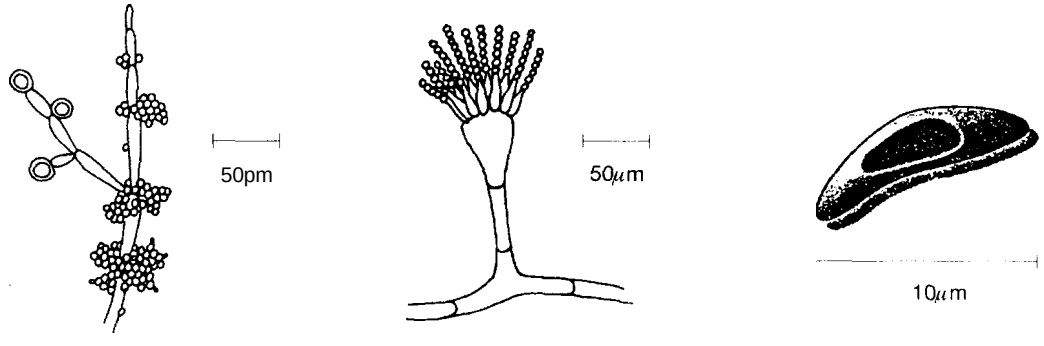
BACTERIA



Staphylococci (food poisoning, toxic shock syndrome) Streptococci (pheumonia, rheumatic fever)

Mycobacterium tuberculosis (tuberculosis) Treponema pallidum (syphilis)

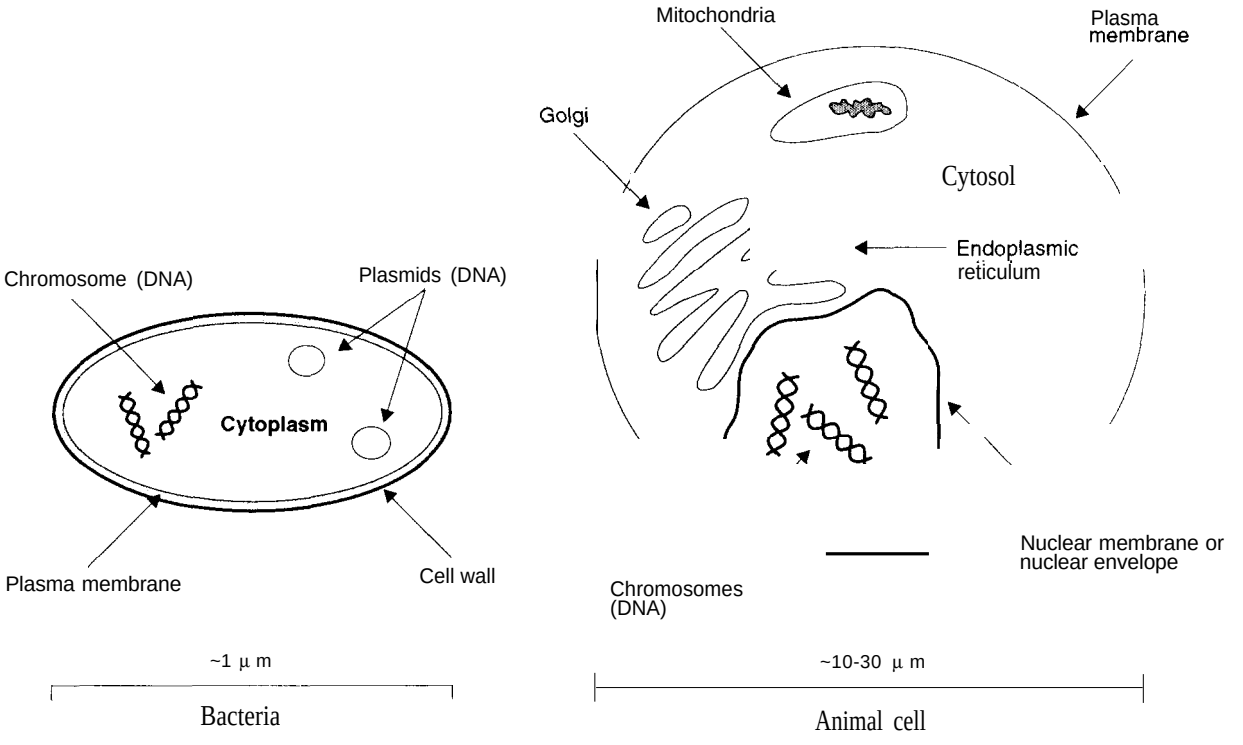
FUNGI PROTOZOA



Candida albicans (yeast infections, thrush) Aspergillus (aspergillosis) Plasmodium (malaria)

SOURCE: Office of Technology Assessment, 1995.

FIGURE 2-2: Simplified Comparison of a Bacterial (Prokaryotic) Cell and an Animal (Eukaryotic) Cell



SOURCE: Office of Technology Assessment, 1995.

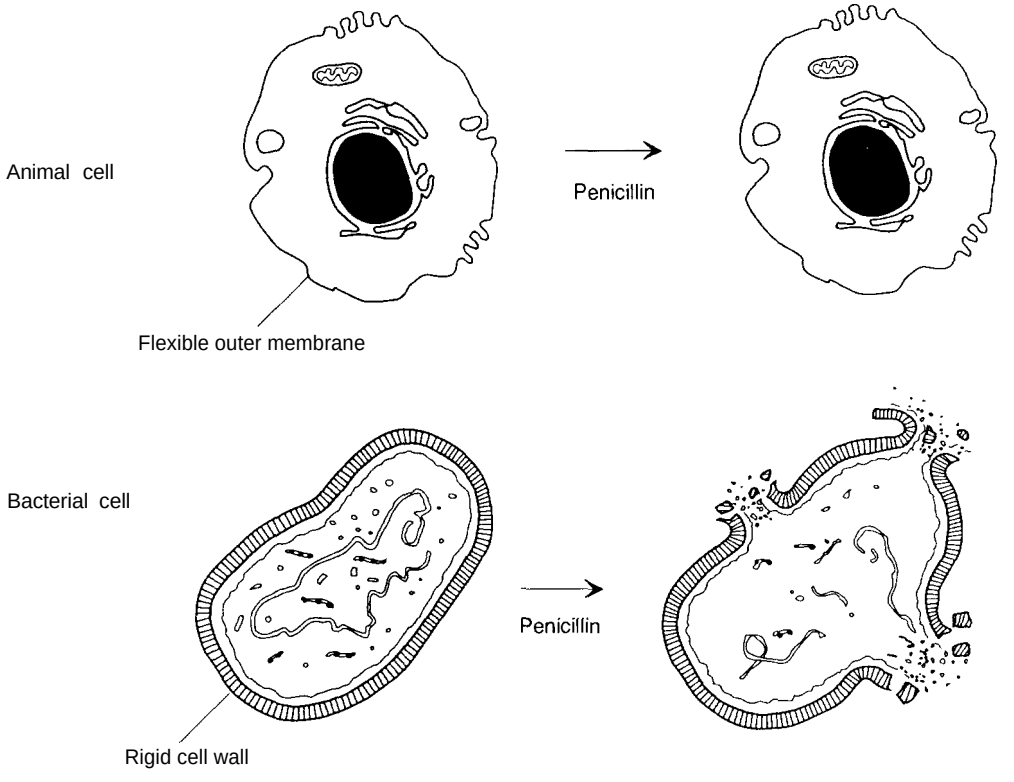
isms. Other chemical agents have been isolated and developed to treat fungal and protozoan infections. Just as with bacteria, which are developing resistance to antibiotics, fungi and protozoae are developing resistance to the drugs used to treat them.

Some bacteria play a role in keeping people healthy. More than 1,000 different species of bacteria normally live benignly in and on the human body. These bacteria, such as *Escherichia coli (see* box 2-1 for a note on bacterial nomenclature) living in the intestine or *Staphylococcus aureus* living on the skin, are called **commensal** organisms. Intestinal bacteria, which are found in concentrations of about 10^{11} (100 billion) bacteria per. gram and account for about 30 percent of the bulk of human feces, produce essential vitamins that are absorbed by the body and provide a barrier against other bacteria becoming established in the intestine. For example, a person may ingest small numbers of a pathogenic Salmonella bacteria but not get sick because the Salmonella is prevented from growing to large numbers by the presence of commensal bacteria in the intestine.

Despite the human body's reliance on bacteria for health, bacteria are far better known as causes of disease. In 1830, infectious diseases caused by bacteria and other microorganisms were a major cause of death, and only 50 percent of the population lived past the age of 25. In the next century, improved sanitation (water purification, sewage systems, pasteurization of milk), general increases in living standards, and the introduction of vaccines reduced the incidence of infectious disease and profoundly changed longevity. By 1935, 50 percent of the population lived past 62 (Schlesinger, 1993).

The capacity of bacteria to cause disease is called **pathogenicity. Virulence** is used as a

FIGURE 2-3: Response of an Animal Cell and a Bacterial Cell to Penicillin



SOURCE: National Institute of General Medical Sciences, Sept. 1993, *Medicines by Design: The Biological Revolution in Pharmacology, NIH* Pub. No, 93-474. Bethesda, MD: National Institutes of Health,

measure of the speed and severity of the resulting disease; more virulent bacteria cause more serious, more rapidly progressing disease, Even commensal bacteria may be harmful under certain conditions. While the skin and mucous membranes normally protect the body from infections, an **opportunistic infection** may result from a bacteria such as S. *aureus* being introduced into the tissues and organs of the body via an open wound, invasive surgery, or use of an invasive device (e.g., a urinary catheter).

Antibiotics often destroy some of the body's commensal bacteria, making way for other infections. For example, the use of some types of antibiotics can allow the organism *Clostridium difficile,* normally present in small numbers in healthy humans, to proliferate and cause the disease pseudomembranous colitis. Yeast infections are common in women treated with antibiotics when antibiotics kill or inhibit commensal bacteria in the vagina. Antibiotics may destroy commensal bacteria in the gut, allowing ingested bacteria, typically resistant to antibiotics, to pervade and cause disease. In two antibiotic-resistant Salmonella outbreaks, it was found that many of the infected people had recently taken antibiotics which may have given the antibiotic-resistant Salmonella an opportunity to become established and cause illness (Holmberg et al., 1984; Spika et al., 1987).

THE DISCOVERY OF ANTIBIOTICS

Before the 1940s, there was little that medicine could do against bacterial infections. Superficial or localized infections could be lanced or surgically opened and cleaned, and locally acting antiseptics could be used to sterilize the area. But once an infection had become "systemic" and

Penicillin-treated bacteria cell bursting at three different places. Photo courtesy of National Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD.

was in the blood stream, little could be done. In World War I, once an infection from even a minor wound developed into dreaded "gas gangrene" (an infection caused by Clostridium bacteria related to the bacteria that cause botulism), there was no treatment except amputation of the wounded limb and prayer that the infection had not reached the soldier's vital organs. People lived in dread that they or their relatives would develop a bacterial pneumonia and die or that a bacterial endocarditis (infection of the heart valves) would doom a child.

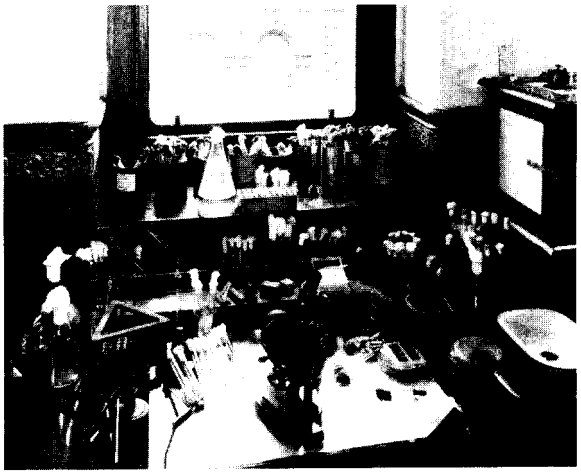
In 1906, chemist Paul Ehrlich provided the first weapon for combating bacterial infection when he discovered that the chemical compound salvarsan was effective against syphilis. In 1936, Gerhard Dogmagk discovered that Prontosil, a synthetic dye, had antibacterial activity. The active chemical component of Prontosil, sulfanilamide, was the first of the sulfonamide (or "sulfa") drugs, and sulfa drugs are still used widely today.

In 1928, Alexander Fleming, an English microbiologist, discovered that a common mold (Penicillium) produced a substance that killed bacteria. Dr. Fleming returned from a weekend

BOX 2-1: Nomenclature

Bacteria and bacterial diseases are our daily companions. There are bacteria literally everywhere in the environment, and a few cause human diseases. Just as in sports where a scorecard is necessary to know the players, some knowledge of bacteria will help the reader, Humans—from the smallest children learning to talk to the astronomer studying craters on other planets—identify and name things So it is with microbiologists who study bacteria and biologists who study other forms of life. Everyone recognizes different mammals—humans, dogs, cats, rats, mice, etc.—and recognizes their unique and salient features if not their scientific *names—Homo sapiens, Canis familiaris, Felis catus, Rattus rattus, Mus muscus.* Such easy familiarity is not possible with organisms that cannot be seen, and everyone has to rely on scientists' identification and nomenclature to talk about bacteria.

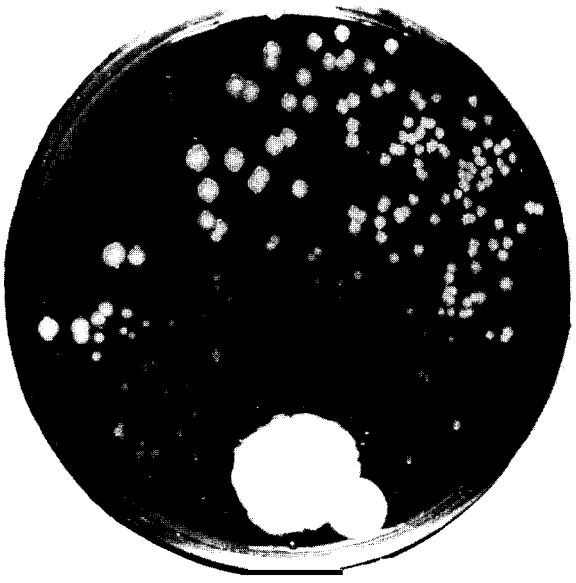
OTA associates bacteria with specific disease states, whenever possible, and uses standard scientific nomenclature. For example, the cause of cholera is *Vibrio cholerae,* where "Vibrio" is the name of a bacterial genus and "cholerae" is the name of a species. After the first use of such a name, the generic name is usually abbreviated, as in *V. cholerae.* When both generic and specific names are used, the words are italicized. When reference is made to a genus, such as "Enterococcus, " the name is capitalized but not italicized, The terms "different bacteria" or "several bacteria" refer to ill-defined collections of different genera. "Strains" refers to further divisions among a species; in particular, there are antibiotic-sensitive strains and antibiotic-resistant strains.



Reconstruction of Fleming's work bench in the room in which penicillin was discovered. St. Mary's Hospital Medical School, Paddington, London, England.

away to his laboratory at St. Mary's Hospital in London and looked at a number of Petri plates that he had seeded with bacteria. The plates had been incubated in his absence and the agar surfaces were sprinkled with colonies of Staphylococcus, a common bacterium frequently found on human skin. Dr. Fleming expected that outcome. One plate was different, however. In addition to the Staphylococcus, there was a large blue-green colony of a common mold called Penicillium. [There's nothing mysterious about the mold. Probably everyone has seen it on an orange that hid itself in the bottom of the refrigerator.] Fleming noted that the Staphylococcus colonies near the mold colony appeared to have dissolved (or "lysed," to use the technical term). He reasoned that the mold was producing and releasing an agent that killed and lysed the bacteria. He called the agent "penicillin." (While the Fleming discovery opened the door to the antibiotics era, there is some circumstantial evidence that people long ago may have benefited from antibiotics; see box 2-2.)

Almost a decade later, at Oxford, a group of researchers and engineers led by H.W. Florey accomplished what Fleming had been unable to do. They scaled up the production of penicillin so that the antibiotic was available in sufficient



Fleming's original culture plate showing fewer and lysed Staphylococcal colonies near the mold. St. Mary's Hospital Medical School, Paddington, London, England.

A production worker pouring penicillin-containing culture medium into a trough for collection in a milk can, mid-1940s. Photo courtesy of The National Museum of American History, Smithsonian Institution, Washington, DC,

BOX 2-2: Antibiotic Use by Ancient Civilizations?

Perhaps unknowingly, earlier civilizations may have benefited from antibiotics. Bassett, Keith, Armelagos, et al. (1980) found evidence for the antibiotic tetracycline in the bones of Nubians who had been buried between 350 and 550 A.D. Streptomycetes, the bacteria from which many antibiotics are derived, are common in the Nubian Sudanese desert, and it is to be expected that the bacteria would have been picked up when the Nubians harvested grain for bread and beer. Conditions in grain storage bins would have favored the growth of the Streptomycetes, which could have been the source of the antibiotic. Drawing upon other information, Bassett et al. state that infectious disease rates were low among this population of Nubians. Regardless of the details, this evidence indicates that humans have interacted with antibiotics from well before 1928.

SOURCE: E.J. Bassett, M.S. Keith, G.J. Armelagos, et al. 1980. "Tetracycline-labeled bone from ancient Sudanese Nubia." *Science* 209:1532-1534.

quantities to be released to the Armed Forces to treat wounded servicemen as well as those with diseases. Early production methods included growing hundreds of cultures of Penicillium in glass bottles (sometimes milk bottles were used), collecting the culture broth, and purifying, concentrating, and packaging the penicillin for shipment. The collection of the penicillin-containing culture medium could be done with devices as simple as a metal trough and a milk can. Currently, the growth (fermentation) of the organisms that produce penicillin and other antibiotics is done in automated factories and with much higher efficiencies than were possible in the 1940s.

By 1944, penicillin supplies were large enough that some of the antibiotic was released for civilian use, and the first antibiotic that could be ingested or injected without toxic side effects entered medical practice. The cover of this report is a reproduction of a 1944 advertisement for penicillin. Penicillin was not made a prescription drug until the 1950s, and, for about a decade, it was available directly to the public (Levy 1992, p. 9).

Other "wonder drugs" followed penicillin, and many dreaded infectious diseases became treatable; people were saved from death and from prolonged periods of disability. Tuberculosis sanatoriums closed because antibiotics were sufficient treatment; people with burns over large areas of their bodies, who would have died in earlier years, survived; childhood meningitis (infections of membranes around the brain or spinal cord), formerly a death sentence, was treatable; prolonged, dangerous, and only-sometimes-effective treatments for syphilis and gonorrhea were replaced by injection or ingestion of an antibiotic. According to Schlessinger (1993), the use of antibiotics, along with nutrition and health education, increased the median lifespan by eight years, from 62 to 70 years, between 1935 and 1955. (There has been little change in median lifespan since 1955.)

The Limits of Antibiotics

Antibiotics can fail to cure an illness because the bacteria are intrinsically **resistant** toward the drugs or because they acquire **resistance**. Resistance is a property of bacteria that confers the capacity to inactivate or exclude antibiotics or a mechanism that blocks the inhibitory or killing effects of antibiotics. Acquired resistance, hereafter simply "resistance," which is characterized by changes in bacteria such that organisms that were formerly treatable with an antibiotic become untreatable, is the focus of this report.

Most bacterial infections can be successfully treated with one antibiotic or another, but the emergence of resistance to older antibiotics, such as penicillin, leads physicians to prescribe newer antibiotics as the first choice in treating many diseases. The use of the newer antibiotic increases selective pressure for the emergence and spread of bacteria resistant to it, and the more an antibiotic is used, the greater the chance that resistance to it will emerge and spread.

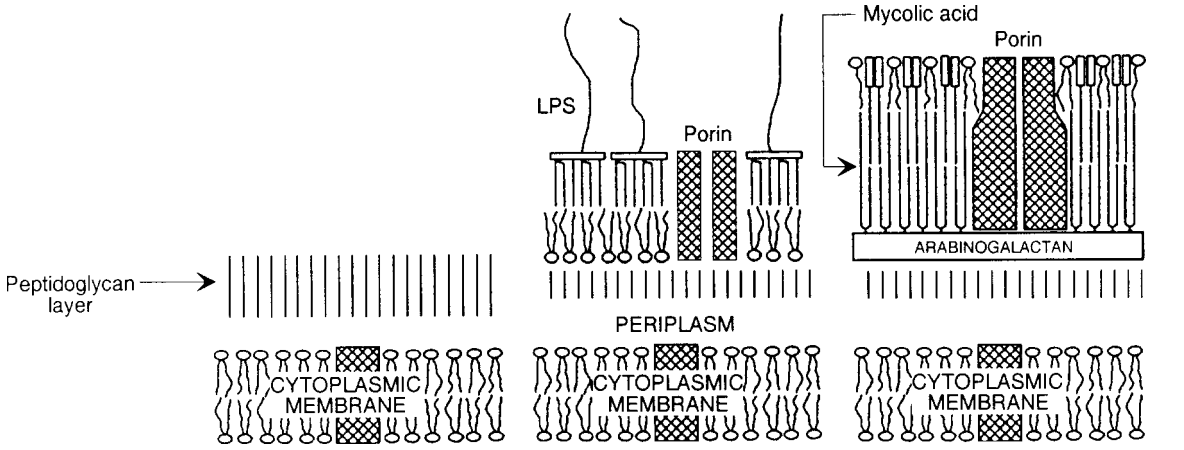
Before turning to the discussion of resistance, some other reasons for treatment failure will be mentioned.

Antibiotics are generally active only against bacteria and not against fungi, protozoa or viruses: Antibiotics act against physiological and biochemical pathways that are specific to bacteria. As already mentioned, antibiotics have few effects in animal and human cells that have biochemical pathways somewhat different from those of bacteria. Other microorganisms, such as fungi (e.g., yeast) and protozoa, also have biochemical pathways different from those of bacteria and, as a result, antibiotics will not work against them. Antibiotics have no effect on viruses because viruses do not have their own biochemistry; they use the biochemical machinery of their host cells that presents no targets for antibiotic action. Despite knowledge that antibiotics work only against bacterial infections, patients request—and physicians prescribe—antibiotics for viral infections, such as the common cold. The consequences of this "inappropriate use" or "overuse" are discussed in chapters 3 and 4.

Some antibiotics are active against only certain kinds of bacteria: There is great diversity among bacteria, and they do not share **all** of the same biochemical and physiological pathways. Therefore, not all antibiotics are active against all bacteria. For example, penicillin works by inhibiting the growth of the bacterial cell wall. Mycobacteria, which are the cause of tuberculosis, do not have the same cell wall structure as other bacteria (figure 2-4), and penicillin will not affect growth of mycobacteria because there is no target for its action.

Mycobacteria walls are a specific example of properties that render some bacteria intrinsically resistant to one or more antibiotics. As a more general example, bacteria are classified as either Gram positive or Gram negative on the basis of their capacity to be colored by a biological stain, and the cell walls of the Gram positives differ from those of the Gram negatives. Some antibiotics are effective against only Gram-positive bacteria, some are effective against only Gram-negative bacteria, and some, the "broad-spectrum antibiotics," are effective against both.

FIGURE 2-4: Cell Envelopes of Bacteria



(Left) Most of the Gram-positive bacteria are covered by a porous peptidoglycan layer, which does not exclude most antimicrobial agents. (Middle) Gram-negative bacteria are surrounded by the outer membrane, which functions as an efficient barrier against many antibiotics. **(Right)** Mycobacteria produce an unusual bilayer, which functions as an exceptionally efficient barrier

SOURCE: H Nikaido, 1994 "Prevention of drug access to bacterial targets: Permeability barriers and active efflux. " *Science* 264:383. Copyright 1994, American Association for the Advancement of Science.

Some bacteria are virulent and can kill quickly: A virulent strain of group A streptococcus causes a disease called toxic shock-like syndrome (TSLS) which killed Muppeteer Jim Henson. Because this strain and other virulent bacteria can "fell otherwise healthy people within hours of the onset of symptoms" (Wright, 1990), antibiotics have to be administered very early in order to defeat the infection.

Some bacteria grow in biofilms that cannot be easily penetrated by antibiotics: Biofilms are multilayer bacterial populations embedded in a film that is attached to some surface. Some examples of bacteria growing in biofilms are the plaque that causes tooth decay, films of *Pseudomonas aeruginosa* that infect lung tissue especially in cystic fibrosis patients, and films that grow on the surfaces of medical devices such as catheters (see chapter 6). Antibiotics often cannot penetrate biofilms; therefore, even though the antibiotic may be effective against the strain of the bacteria in the laboratory, the antibiotic may be ineffective against the infection.

Mechanisms for the Emergence and Spread of Resistance

When a new antibiotic is introduced, many bacteria are susceptible to it. Hughes and Datta (1983) demonstrated that bacteria preserved from 1917-1954 (the "pre-antibiotic" era) had little if any antibiotic resistance except intrinsic resistance. However, since the dawn of the antibiotic age, acquired resistance to every known antibiotic has been observed in one or more bacterial strains. This resistance sometimes arises in an individual patient during the course of treatment, but more often people are infected by resistant bacteria that are acquired from the community or the hospital environment.

Mutations

Antibiotic resistance arises through processes that involve mutations and selection. Mutations occur spontaneously in bacterial DNA that modify or eliminate a target for an antibiotic's action, or that cause changes in the bacteria surface so that the antibiotic is not taken up, or that cause the production of an enzyme that inactivates the antibiotic, or that cause the antibiotic to be excreted from the bacterial cell. These mutations happen in the absence of any exposure to antibiotics, but the presence of an antibiotic favors the growth of the bacteria that contain a mutation for resistance, or in the usual jargon, the antibiotic "selects for" the mutant bacteria. Weiner (1995 at pp. 257-262) discusses the origins of mutations to antibiotic resistance and the selection of those mutations in an evolutionary context.

Mutations are of three general kinds. **Point mutations** are "single letter" mistakes that occasionally occur in copying the DNA code, and they can cause a small change in an enzyme or structural protein. The other two kinds of mutations, **insertions** and **deletions**, generally have more far-reaching effects; they can completely eliminate an enzyme activity or destroy a structural protein. Mutations are passed on to future generations of bacteria, and the number of resistant bacteria can increase very rapidly. Under the most favorable conditions, some bacteria can duplicate every 20 minutes.

As shown on figure 2-2, bacterial DNA is present on "chromosomes" and "plasmids." Chromosomes usually contain all the genes necessary for the life of the bacteria, and some genes that confer resistance to antibiotics are found on the chromosome. Plasmids, smaller pieces of DNA that replicate separately from the chromosome, can also be present. They can and often do carry genes for antibiotic resistance, and, as discussed below, they can be transferred from bacterium to bacterium.

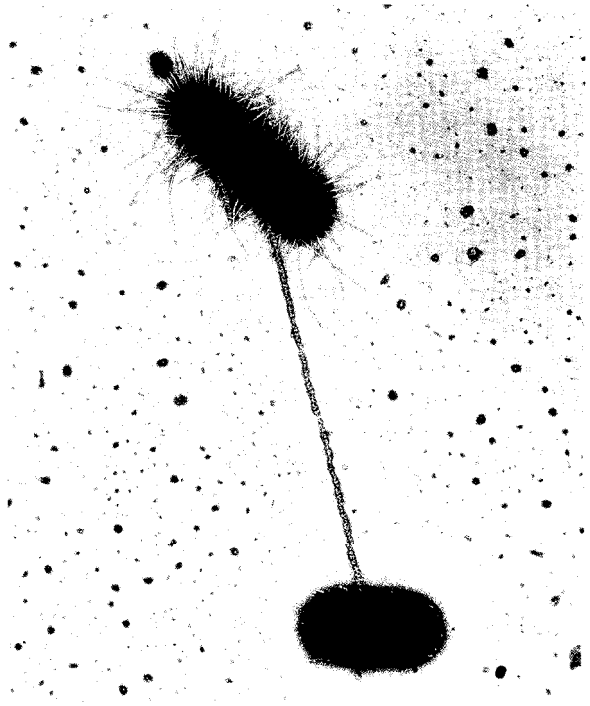
Chromosomal mutations

Genes for resistance to fluoroquinolone antibiotics (e.g., ciprofloxacin and ofloxacin) are known to occur, so far, only on chromosomes and not on plasmids. Single courses of therapy with fluoroquinolones may produce only low levels of resistance, but multiple mutations selected by repeated exposure to increasing doses of fluoroquinolones can confer high levels of resistance (Hooper and Wolfson, 1991). Even though muta-

tions occur only rarely, prolonged exposures to antibiotics can select for those mutations during a patient's treatment. In a study of 28 cystic fibrosis patients with chronic broncho-pulmonary *P. aeruginosa* infections treated with 14-day regimens of ciprofloxacin or ofloxacin, one developed resistance resulting in treatment failure, three developed intermediate resistance, and six developed low levels of resistance (Jensen et al., 1987). Three months after the end of treatment, the average resistance of the patients' *P. aeruginosa* to ciprofloxacin or ofloxacin remained somewhat higher than before treatment. Similarly, Chow et al. (1991) observed the development of antibiotic resistance in strains of Enterobacter during therapy.

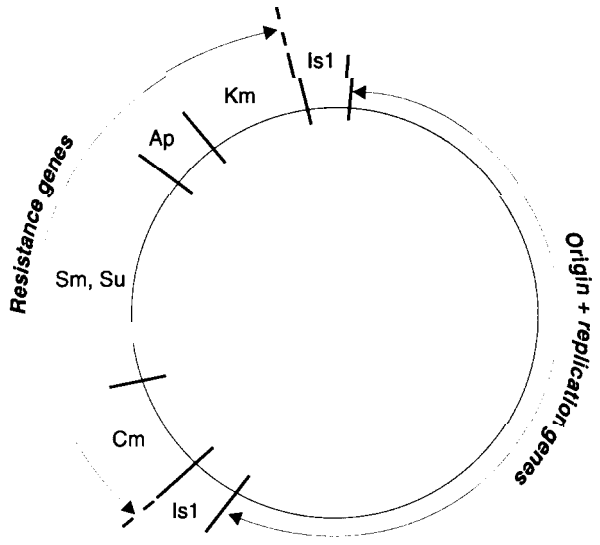
Plasmids and gene transfer

Plasmids are able to pass directly between bacteria through the process of **conjugation,** in which a newly replicated plasmid is transferred from the donor cell to the recipient cell through a pilus or conjugation tube. When the process is complete, both bacteria contain a copy of the plasmid, and both have the capacity to replicate and transfer the plasmid.



A Micrograph of conjugation between two bacteria. Photo courtesy of Dr. Charles Brinton, University of Pittsburgh.

FIGURE 2-5: Genetic Map of a Plasmid



Cm Chloramphenicol
Su Sulfonamide
Sm Streptomycin
Ap Ampicillin
Km Kanamycin
Is1 Transposon insertion factor

SOURCE: Office of Technology Assessment, 1995.

Plasmids can recombine with DNA from other plasmids, and that process can produce a single plasmid that carries multiple genes for resistance to different antibiotics (Condit and Levin 1990). This has important clinical consequences because the use of any one of the antibiotics shown in figure 2-5 could select for the plasmid that contains genes for resistance to all the antibiotics shown there.

Scientists confirmed the role of plasmids and conjugation in spreading antibiotic resistance during a dysentery epidemic in Japan in the late 1950s (Watanabe, 1963). The epidemic was characterized by increasing numbers of *Shigella dysenteriae* strains that were resistant to as many as four antibiotics simultaneously. Such bacteria became so frequent that health officials concluded that their emergence could not be attributed to repeated mutations arising in one bacterium after another because mutations occur

too rarely. Scientists showed that conjugational transfer of multiple-resistant plasmids accounted for the epidemic and established plasmids as major agents in the spread of antibiotic-resistant genes.

Hughes and Datta (1983), who examined preserved bacterial strains from the pre-antibiotic era, showed that plasmids were present in many of the bacteria and that 24 percent of the plasmids were able to be transferred by conjugation between bacteria. However, very few of the preserved bacteria were resistant to antibiotics and those few were resistant to only one antibiotic. This indicates that multi-resistance plasmids must have been created in the decades following the discovery of penicillin, when the use of antibiotics became extensive. Importantly, however, the pre-existing transferable plasmids in bacteria became the vehicle for transfer of multiple antibiotic-resistant genes.

Resistance genes can also travel on **transposons**, small pieces of DNA that can transfer to different sites on bacterial chromosomes and plasmids in the same bacterial cell or in different bacterial cells. Hall and coworkers (Hall and Stokes, 1993) have been studying the structure of some transposons called **integrons** that carry antibiotic-resistance genes. The integrons are like freight trains: sequences of DNA necessary for the functioning of the integrons at the front and the back are like the engine and the caboose, and any number of "cassettes" of resistance genes, like the cars of the train, can be carried between them. Different cassettes can insert into integrons, and this facilitates the acquisition of resistance genes by bacteria. Collis and Hall (1995) have also found that the **expression** of the integrons depends on their position in the cassette: resistance coded by genes close to the front of the train is stronger than resistance from genes near the back of the train. This helps explain the variability in the levels of resistance between different strains of bacteria.

The origin of the resistance genes that can be transferred between bacteria on plasmids and transposons is unknown, but some, at least, might have originated as a self-protective mechanism in antibiotic-producing organisms. For example, some strains of streptomyces that produce aminoglycosides (streptomycin is an aminoglycoside) also produce aminoglycoside-modifying enzymes (Benveniste and Davies, 1973).

Genes can be transferred between different species of bacteria. In a 1979 outbreak in a Kentucky hospital (Tauxe, Holmberg, and Cohen, 1989), 31 patients and personnel became infected with a strain of *Staph. aureus* that was resistant to methicillin, penicillin, gentamicin, erythromycin, clindamycin and tetracycline. Bacteria isolated from all of those affected contained the same resistance plasmid. Plasmids of a similar size were also found in the common skin commensal organism *Staph. epidermis* from the affected patients. Analysis of the plasmids by molecular techniques suggested that the same plasmid had been transferred between *Staph. aureus* and *Staph. epidermis*.

In another study that demonstrated inter-species transfer, Tauxe, Cavanagh, and Cohen (1989) examined multiple-antibiotic-resistant *E. coli* and *Shigella flexneri* that were isolated from a hospitalized patient. Their analysis indicated that the resistant genes had been transferred from the *E. coli* to the *S. flexneri* and that the antibiotic-resistant *S. flexneri* had then become the cause of a small outbreak of infections in the community. These examples show that resistance genes can be transferred between different bacterial species and demonstrate a pathway for widespread distribution of antibiotic-resistant genes.

There are two other mechanisms for gene transfer in addition to conjugation: **transduction** and **transformation**. In transduction, genes are transferred by bacterial viruses (called "bacteriophages" or "phages"). In transformation, pieces of DNA in the bacteria's environment are taken into the bacteria and incorporated into the bacterial chromosome. *Hemophilus influenzae* takes up DNA from its surroundings, and recently reported data indicate that transformation may play an important role in the survival of those bacteria (box 2-3).

BOX 2-3: The Complete DNA Sequence of *Haemophilus influenzae*

Using a variety of newly discovered methods, scientists have been working to sequence the DNA of several different organisms, from humans to mice to bacteria. These sequences, when complete, locate every "base" or "nucleotide," the chemical units that carry the genetic code in an organism's genome.

H.O. Smith and J.C. Venter led a group of scientists who completely mapped the DNA sequence of *Haemophilus influenzae* Rd (Fleischmann et al., 1995). Their success marked the first complete DNA sequence for any free-living organism, and Venter has announced that sequences for two other bacteria are nearly completed (Nowak, 1995).

The speed at which these sequences can be completed opens up a new era in understanding how bacterial DNA directs the activity of bacterial metabolism, and, in particular, it will enable scientists to understand the genes that are involved in virulence. For instance, *H. influenzae* Rb is a non-pathogenic "laboratory strain" which is closely related to the human pathogen *H. influenzae* b. By comparing the DNA sequences from the Rb and b strains of *H. influenzae*, Fleischmann and colleagues (1995) were able to demonstrate that eight genes that code for proteins necessary for the b strain to adhere to host cells were missing from the Rb strain. This suggests that the Rb strains may not be pathogenic, at least in part, because they cannot attach firmly to host cells.

H. influenzae can take up DNA from its environment and recombine the taken-up DNA into its own DNA through the process called transformation. Smith et al. (1995) found that certain DNA sequences occur at 1,465 different locations on the *H. influenzae* DNA and that these sequences cause the bacteria to preferentially take up and incorporate DNA from its own species.

This feature enhances the capacity of *H. influenzae* to take up DNA from other *H. influenzae* that have died. Why it would be desirable to take up DNA from bacteria that have been killed is unclear; presumably, the bacteria that die were less fit for their environment. However, the fact that the bacteria have so many recognition sequences suggests that the sequences, which increase opportunities for recombination between the DNA of the dead bacteria and the surviving bacteria, are of survival advantage to the bacteria.

SOURCES: R.D. Fleischmann, M.D. Adams, O. White, O., et al. 1995. " Whole-genome random sequencing and assembly of Haemophilus influenzae Rd." *Science* 269:496-512; Nowak, R. 1995. "Bacterial genome sequence bagged." *Science* 269:468-470; H.O. Smith, J.-F. Tomb, B.A. Dougherty, et al. 1995. "Frequency and distribution of DNA uptake signal sequences in the *Haemophilus influenzae* Rd genome." *Science* 269:538-540.

International Spread of Antibiotic Resistance

Antibiotic-resistance genes move with travelers from one country to another, making antibiotic resistance an international problem. O'Brien et al. (1985) document the intercontinental spread of an antibiotic-resistant gene on a plasmid, and Soares et al. (1992) reported the introduction of strains of multiple-resistant *Streptococcus pneumoniae* from Spain to Iceland in the late 1980s. These examples illustrate that antibiotic use and bacterial resistance patterns all over the world will have an impact on the United States and indicate the importance of international cooperation in dealing with the antibiotic-resistance problem.

Persistence of Antibiotic Resistance Genes in the Absence of Antibiotics

The extent to which antibiotic resistance can be controlled by limiting the use of antibiotics may be answered by studying the molecular mechanisms of transposon and plasmid replication and the behavior of populations of bacteria. Antibiotic use selects for bacteria that carry antibiotic-resistance genes, but the resistant bacteria might be less efficient or use more energy because they carry "excess baggage" of altered or extra genes.

Such genes can make the difference between survival and death in the presence of an antibiotic, but their maintenance in an antibiotic-free environment might put bacteria that bear them at a competitive disadvantage with bacteria that do not carry such genes.

Simonsen (1991) discusses the fate of plasmids in the absence of selection pressure from antibiotics. The "excess baggage" theory predicts that easing the selective pressure by decreasing the use of antibiotics would lead to a decrease in the carriage of antibiotic-resistance genes by bacteria. But Bouma and Lenski (1988) showed that this may not always be the case. They inserted a plasmid that carried a tetracycline-resistance gene into a strain of *E. coli*. The *E. coli* carrying the plasmid grew poorly as compared to *E. coli* without it (the plasmid is "excess baggage"). Of course, in the presence of tetracycline, the bacteria that did not have the plasmid would not grow. As expected, after 500 generations of growth in tetracycline, all bacteria contained the plasmid. Moreover, even in the absence of tetracycline, the plasmid-bearing bacteria now grew better than the bacteria without the plasmid. The bacteria had somehow adapted in those 500 generations to become more efficient while retaining the plasmid.

This result leads to the suggestion that evolution can produce plasmid-carrying bacteria that are not at significant disadvantage in competition with other bacteria in antibiotic-free environments. It can also be interpreted to indicate that plasmid-carrying bacteria will not be eliminated by eliminating antibiotics.

On the other hand, there are many examples in which controlling the use of antibiotics leads to a decrease in the frequency of bacteria carrying antibiotic-resistance genes. This may reflect that antibiotic-susceptible bacteria (those without "excess baggage") usually outgrow antibiotic-resistant bacteria so that the resistant bacteria become a smaller and smaller proportion of the total population. However, this process may be very slow, and the resistance does not decrease to zero. The observation that the antibiotic-resistant bacteria do not disappear (drop to zero) may be consistent with the results of Bouma and Lenski, because bacteria may adapt so that carrying plasmids containing resistance genes provides an advantage, even in the absence of the antibiotic.

CONFRONTING ANTIBIOTIC RESISTANCE

Currently, half a century after the introduction of "wonder drugs," scientists, physicians and the public fear the re-emergence of infectious diseases caused by antibiotic-resistant bacteria. Krause (1992) observed

> [M]icrobes are not idle bystanders, waiting for new opportunities offered by human mobility, ignorance or neglect. Microbes possess remarkable genetic versatility that enables them to develop new pathogenic vigor, to escape population immunity by acquiring new antigens, and to develop antibiotic resistance.

Scientists who contributed to the biological research that produced antibiotics warn that society has unwisely tolerated the risk that was evident in reports of the proliferation of genetic alterations in bacteria that spread antibiotic resistance:

> The stunning success of the pharmaceutical industry in the United States, Japan, the United Kingdom, France and Germany in creating new antibiotics over the past three decades has caused society and the scientific community to become complacent about the potential of bacterial resistance... [D]espite all these antibiotics, a person could die in a hospital in New York, San Francisco, Paris, Barcelona, Tokyo, or Singapore as a result of a resistant bacterial infection (Neu, 1992).

There are many questions surrounding antibiotic resistance. Is it possible that alternative strategies of scientific research and antibiotic development could have prevented this outcome? Have antibiotics been improperly prescribed or inappropriately requested by patients? If evidence was available from the start that disease-carrying bacteria could become resistant to antibiotics, what postponed the crisis for 50 years? Although the Institute of Medicine identified antibiotic-resistant microorganisms as only one of six factors contributing to the rising risk

of morbidity and mortality from infection, it warned that antibiotic resistance "may be a greater threat to the public than the emergence of a new disease" (IOM, 1992).

The following chapters discuss what is known about antibiotic resistance and address the important questions of what can be done now to help slow the emergence and spread of antibiotic-resistant bacteria, to preserve the capacity to treat bacterial infectious diseases with available antibiotics, and to develop new antibiotics.

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