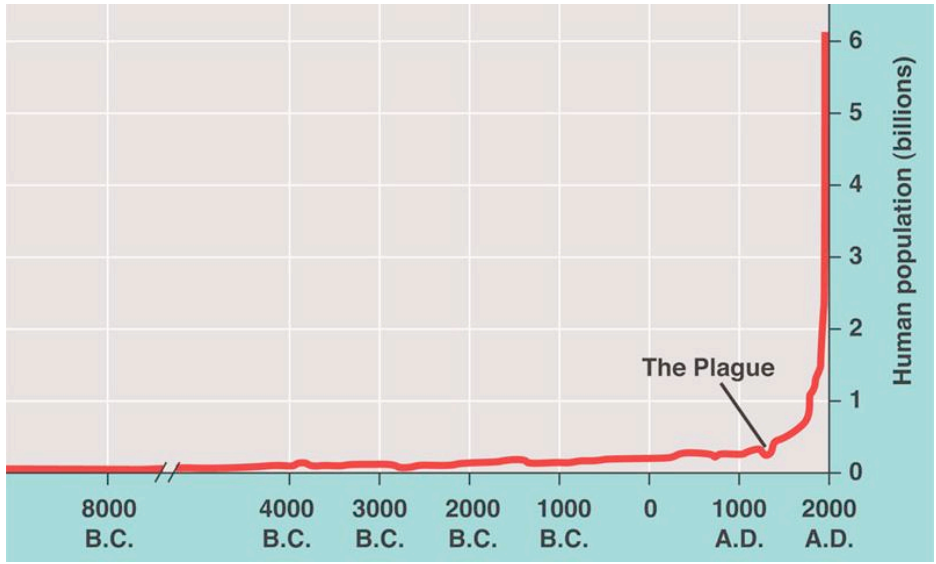
Antibiotics & Antibiotic Resistance

1-18-07

The Lethal Gifts of Agriculture

- **Agriculture did not itself create new infections; it simply accentuated those that were already present.**
- **Zoonotic diseases: dogs (65), cattle (45), sheep and goats (46), pigs (42), horses (35), rats (32), and poultry (26).**



Agricultural revolution begins

Magic Bullets, Miracle Drugs

- **When first introduced, they were regarded as miraculous compounds**
- **Most people today have lost this earlier awe of antibiotics.**
- **Sadly, we have come to take them for granted.**
  - **In fact, we take antibiotics so much for granted that many of us willingly participate in their misuse and overuse.**

Examples of Misuse

- **Used to treat viral infections.**
  - **common cold**
  - **flu**
- **Physicians know that using antibiotics for this purpose is not good medical practice, but many yield to pressure from patients who are too sick and too concerned with job and family pressures to care much about the antibiotic is appropriate.**
- **‘The antibiotic can’t hurt and it might help.’**

Consequences of Misuse

- **May not cause immediate damage to the patient being treated.**
  - **allergy**
- **Dangerous to the population as a whole.**
- **Overuse leads to reduction in the effectiveness of the antibiotic.**
  - **increases the selection for bacteria that have become resistant to it.**

What are they?

- **Can we define them as chemical compounds that kill or inhibit the growth of bacteria?**
- **This definition is a bit misleading.**
  - **Many compounds such as arsenic or mercury can kill bacteria, but few people would volunteer to take large doses of arsenic orally or by injection.**
  - **So what is the difference?**

The revolutionary feature of antibiotics, compared to earlier therapies for infectious disease, is that they are designed to be devastating for bacteria but to have few if any adverse effects on the human body.

Magic Bullets

Where do they come from?

- **Produced by bacteria or fungi.**
  - **‘biotic’ referring to their biologic origin.**
- **Synthetically generated antibiotics technically should be referred to as antibacterials.**
- **We will use the term *antibiotic*.**

How are antibiotics different from disinfectants and vaccines?

- **Antiseptics**
  - too toxic for internal use
  - can be applied to skin
    - *e.g.*, peroxide, triclosan, mercurochrome
- **Disinfectants**
  - too toxic for skin
  - effective for cleaning inanimate objects
  - *e.g.*, bleach, formaldehyde
- **Vaccines**
  - prime the immune system
  - take time
  - preventive

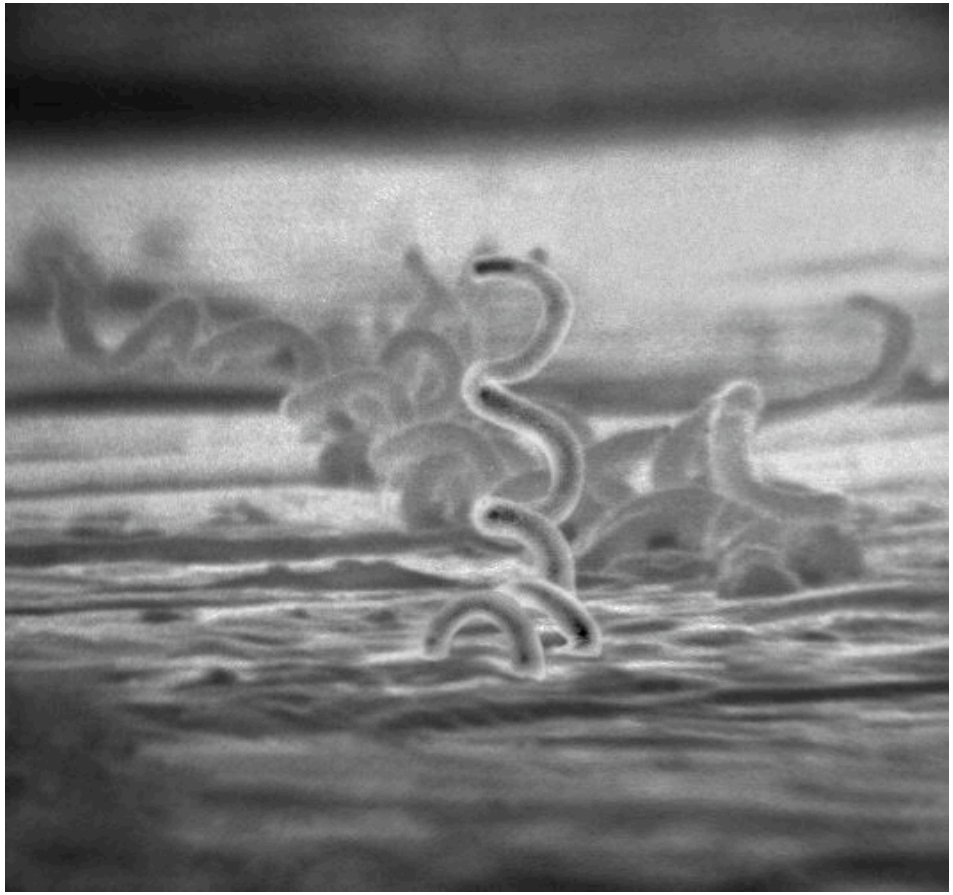
The ‘miracle’ in ‘miracle drugs’

When antibiotics first appeared in the 1930s and 1940s, people held them in greater awe than we do today.

Lets take a look to see why...

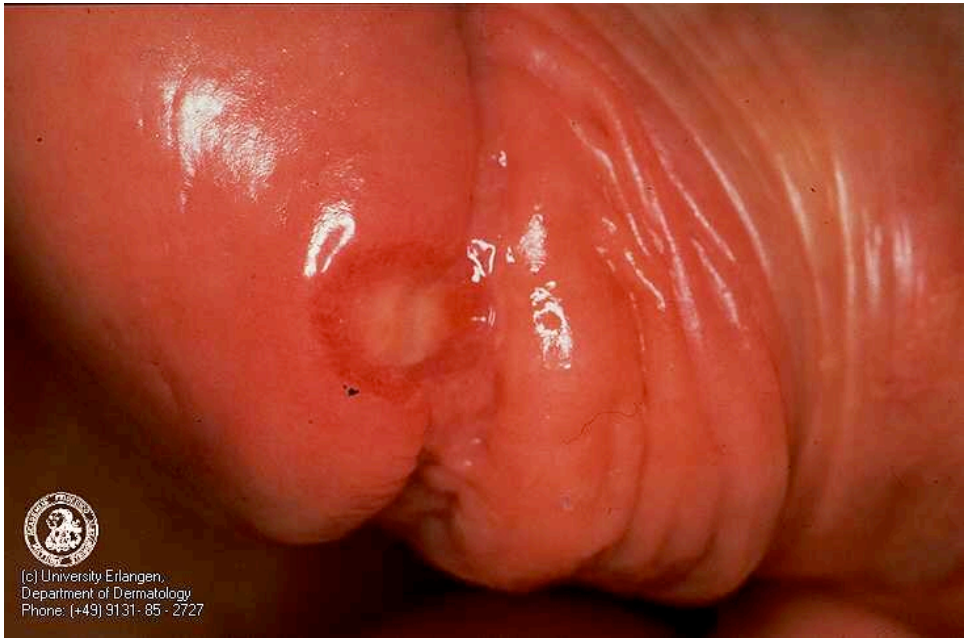
Syphilis

- **Caused by the spirochete *Treponema pallidum*.**
- **Gram negative.**
- **To date, can not be grown in culture.**
  - **‘Trep’-corckscrew**
  - **‘nema’-thread**
  - **‘pallidum’-pale**



Pre-20th Century

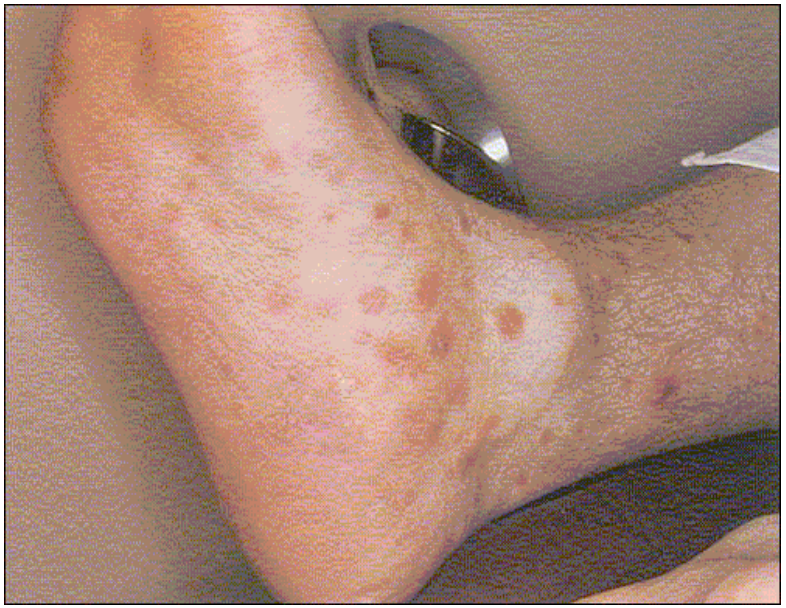
- **If you were a sexually active man or woman in the pre-20th century U. S. or European city, syphilis was a very real threat.**
- **Untreated syphilis progresses through 3 stages:**
  - **stage 1-bacterial growth at the site of infection.**
  - **chancre**
  - **as awful as it looks, it is not painful...**
  - **heals in 1-2 weeks...false sense of security.**



Primary lesion

Stage 2 Syphilis

- **Bacteria enter blood stream.**
- **Rash appears on the palms or feet.**
- **Clears up like the chancre even though bacteria still present.**
- ***T. pallidum* move out of the blood stream into tissues (as a result of immunity.**
- **Lay dormant for months or years.**
- **A painful death will ensue.**



Origin of the Word

- **The word itself (Syphilis) was coined by Girolamo Fracastoro, an Italian poet and physician.**
- **He published a poem called '*Syphilis, sive Morbus Gallicus*,' which translates as 'Syphilis, or the French Disease.'**
- **First proponent of the 'Germ Theory.'**

1478-1553


Girolamo Fracastoro
1478 – 1553
0,77 €
SAN MARINO

Syphilis, sive Morbus Gallicus

- In the poem, a shepherd named Syphilus is supposed to have been the first victim of the disease.
- The original source of the name Syphilus is uncertain but may have come from the poetry of Ovid.
  - **Bottom line was that the Italians blamed the French for syphilis.**
  - **The French blamed the Italians.**
  - **In Spain, the disease was blamed on the Jews, who had been forced out of Spain, also in that memorable year of 1492.**
  - **Russians called it 'the Polish disease.'**
  - **The Japanese called it 'the Chinese disease.'**
  - **The English called it 'the Spanish disease.'**

Did Columbus's men bring syphilis back to Europe?

- **"Contemporaries thought it was a new disease against which Eurasian populations had no established immunities. The timing of the first outbreak of syphilis in Europe and the place where it occurred certainly seems to fit what one would expect of the disease had it been imported from America by Columbus' returning sailors. This theory...became almost universally accepted...until recently."**
  - **W. H. McNeill. *Plagues and People*. 1977**
  - **This urban legend acquired a sort of mystique as an unintended form of 'revenge' unwittingly exacted by the Indians for what Columbus and the the arrival of the Europeans had done to them.**

Evidence against Columbus

- **Pre-Columbian syphilis in the Old World.**
  - **245 skeletons recently unearthed from a medieval monastery known as Blackfriars (Hull, England) showed distinctive signs of syphilis (1300-1420).**
  - **Notched teeth from the remains of individuals from the port city of Metaponto, Italy (600 B.C.).**
  - **Also in remains from those who died in Pompeii following the eruption of Mount Vesuvius (79 A.D.).**
    - **Some believe syphilis has been in Europe for thousands of years.**

Notched teeth & caries sicca

The Beginning of the Outbreak

- **The return of Columbus and his men coincided with a massive outbreak of syphilis in Europe.**
- **Syphilis in epidemic proportions 1st appeared during a war being fought in Naples in 1494.**
- **The army of the French king, Charles the VIII, withdrew from Naples, and the disease was soon spreading throughout Europe.**

The spread of syphilis--'The Great Pox'

- **The mercenaries of Charles VIII spread the disease over Europe.**
  - **Some of these mercenaries joined Perkin Warbeck in Scotland and, with the support of James IV, invaded England. The pox was evident in the invading troops.**
  - **Hungary and Russia by 1497**
  - **Africa and the Middle East by 1498**
  - **The Portuguese carried it around the Cape of Good Hope (Vasco de Gama) to India in1498.**
  - **China by 1505.**
  - **Australia by 1515.**
  - **Japan by 1569.**

The spread of syphilis--'The Great Pox'

- **European sailors carried the Great Pox to every continent save for Antarctica.**
- **Syphilis was so ubiquitous by the 19th century that it could be considered to be the AIDS epidemic of that era.**

- **The parallel between AIDS and syphilis does not end there.**

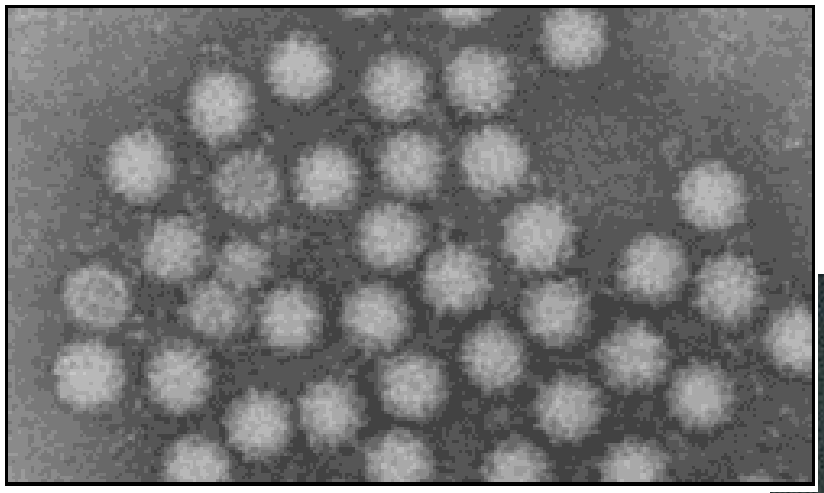
Progression of symptoms and Severity

- **Favoring the theory that syphilis was a new disease was its severity during the beginning years of the outbreak.**
  - **From 1494-1516 the symptoms were described as genital ulcers, followed by a rash, and then the disease spread throughout the body, affecting the gums, palate, uvula, jaw, and tonsils eventually destroying these organs.**
    - **the victims suffered pains in the muscles and there was early death--an acute disease.**

Progression of Symptoms & Severity

- From 1516-1526, two new symptoms were added to the list: bone inflammation and hard pustules.
- From 1526-1560, the severity of symptoms diminished, and thereafter its lethal effects continued to decline but...
- From 1560-1610 there was another symptom: ringing in the ears.
- In the 1700s, syphilis was a dangerous but not an explosive infection.
- By the 1800s, both the virulence of the pathogen and the number of cases declined.
- Even so, by the end of the 19th century, 10% of the European population was infected.
- Early 20th century, 1/3 of the patients in mental institutions could trace their neurological symptoms to syphilis.

Myxoma virus and Rabbits



Pre-Columbian Origin

- **All of the treponemes that cause the human diseases yaws, pinta, and syphilis are identical in their morphology.**
- **Genomes contain only 1000 genes.**
- **No gene(s) has been found to be specifically associated with virulence.**
- **Only symptoms differ.**

Pre-Columbian Origin

- **This theory suggests that human treponemes may have come from animals and that an infection similar to pinta (*T. carateum*).**
- **Localized in the skin.**
- **Arose about 15,000 B.C. in Africa.**
- **With human migration passed across the Bering Straits it became isolated in the tropics of the Americas.**
- **Pinta only found in skin--disseminated by introduction into skin.**

Pre-Columbian Origin

- **Around 10,000 B.C., the pinta-causing spirochete mutated into a pathogen causing a disease similar to yaws.**
  - ***T. pallidum pertenue***
- **Restricted to tropical areas of Africa.**
- **Brought to the Americas by the slave trade.**
- **Yaws exists today in tropical climates.**
- **Not benign.**
- **Causes disfigurement.**

Pre-Columbian Origin

- **As populations of *Homo sapiens* began to penetrate into temperate and drier regions, the presence of cooler climate provoked another mutation, allowing the bacteria to colonize the throat and to produce skin lesions similar to those of endemic syphilis.**
  - ***T. pallidum endemicum***
- **Transmission occurs via contaminated objects (drinking vessels, utensils, saliva, mouth-to-mouth).**
- **It has been known to exist in Africa for centuries.**
- **Today found in pre-pubescent children living under semi-nomadic conditions in the northern Sahara, southwest Asia and Australia.**

Pre-Columbian Origin

- A third mutation resulted in the true veneral transmission form of spirochetes.
- Coincided with the emergence off cities about 3000 B.C. in the Middle East.
- Spread to the Mediterranean in mild form.
- Remained endemic for centuries.
- Fourth mutation occurred in Europe in the 15th century
  - environmental conditions, behavioral patterns allowed the veneral form of the disease to emerge.

Syphilis and Its Social Context

- Charles VIII
- Francis I
- Pope Alexander Borgia
- Benvenuto Cellini
- Henri Toulouse-Lautrec
- Heinrich Hinze
- Franz Schubert
- Peter the Great
- Catherine the Great
- Florence Nightingale
- John Keats
- Guy de Maupassant
- Al Capone
- Randolph Churchill
- Beethoven's deafness?

Syphilis and Its Social Context

- **Ivan, Grand Duke of Muscovy, born 1530.**
- **In 1552 wife Anastasia game birth to a son, Dimitri, who died at age 6 months of congenital syphilis.**
- **9 months later Ivan was born and in 1558 a third son, Fedor, was born.**

Syphilis and Its Social Context

- **It was suspected that Ivan was infected with syphilis prior to his marriage.**
- **Anastasia died in 1560 and Ivan remarried in 1561.**
- **She gave birth to Vassili, who lived 5 weeks.**
- **By 1563, there was evidence that Ivan was suffering from neurosyphilis.**
- **From 1565-1584, Ivan engaged in a reign of terror.**

Syphilis and Its Social Context

- **Ivan tortured, flogged, burned, and boiled those he considered to be his enemies.**
- **Claiming conspiracy, he had thousands of citizens of Novgorod flogged to death, roasted alive, or drowned under the ice.**
- **Ivan and his son raped the widow and daughter of Prince Viskavati (whom they hanged).**
- **In 1581, during a fit of rage, he stabbed his son to death.**
- **Ivan died a gibbering idiot at 54.**
- **This left the throne to the congenital idiot Fedor.**

Toombs of Ivan the Terrible and his sons
Ivan and Fyodor

Henry the VIII and the Decline of the Tudor Line

- Catherine of Aragon → -Birth to a child who died within days.
  -She had 3 more stillborns.
- Anne Boleyn → Miscarried on child at 6 months and another at 3.5 months.
- Jane Seymour → One son Edward who died of 15(?).
- Elizabeth I (born of Boleyn)
- Marry Tudor → Also nearsighted, deaf, large flat nose that discharged a foul smelling pus

The man himself...Henry the VIII

- **Became sterile or impotent in his late forties.**
- **His character (like Ivan) began to change.**
- **Suffered from ulcers, headaches, sore throats.**
- **Had a gumma on his nose.**
- **Bent on terror and slaughtered the Lollards, Lutherans, Anabaptists, and Catholics.**

“The inability of royal and aristocratic leaders to give birth to healthy children accelerated social mobility making more room at the top than there would have been otherwise--thanks to syphilis!”

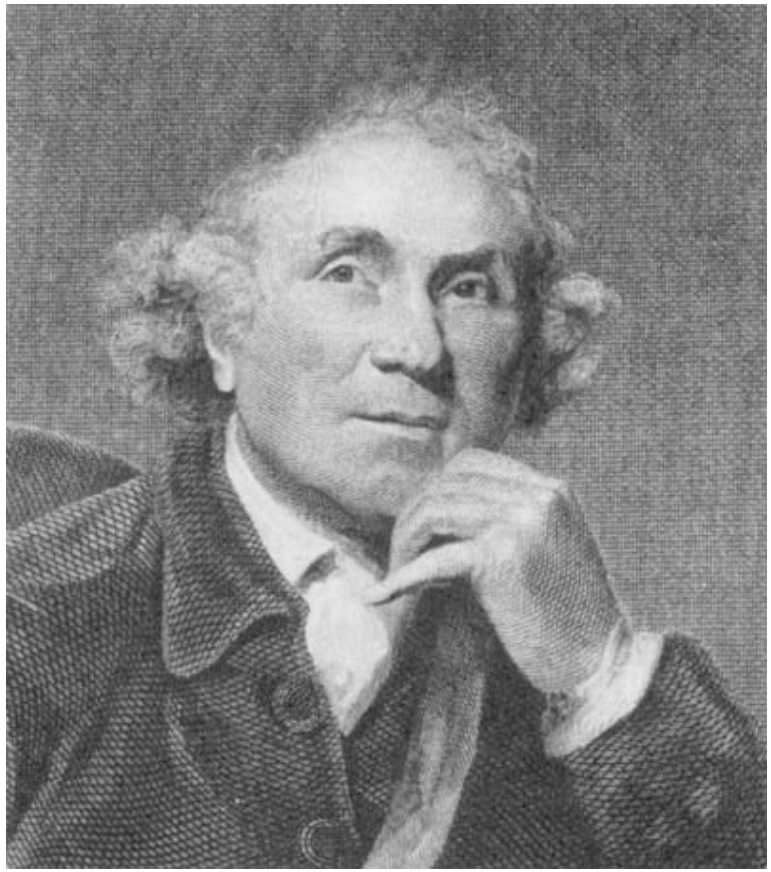
William H. McNeill

Spirochete Discovered

- **One of the reasons it took so long for microbe hunters to identify the cause of syphilis was that it was confused and associated with another STI, gonorrhea.**
- **Even the great anatomist and physician John Hunter (1748), of St. George's hospital in London, could not solve the puzzle...**

John Hunter (1728-1793)

- **Considered one of the greatest anatomists of all time and the founder of experimental pathology in England. Hunter put the practice of surgery on a scientific foundation and laid the framework for the twentieth century developments. His saying "Don't think, try the experiment" has inspired generations of modern surgeons.**



The opposite of Jenner...

- **No vaccine available.**
- **An early disastrous attempt by Joseph Auzias-Turenne (1812-1870) drew inspiration from Jenner's small pox vaccine.**
- **He took a part of a soft chancre and prepared it for use as vaccine.**
- **The chancre was caused by *H. ducreyi*.**
- **Had the scheme prevailed in 19th century France, thousands would have been mistakenly believed to have been vaccinated against syphilis; instead, they would have gotten chancroid!**

Syphilis Cure

- **Moralists living at the time when syphilis ran its course unimpeded would have disapproved of a cure for syphilis.**
  - **'Wages of sin.'**
- **What about unsuspecting spouses or fetuses?**
- **What about present time? Who worries about syphilis? How would antibiotics have changed history?**

The Wages of Sin: Sex and Disease, Past and Present--Peter Allen

- **15th and 16th century treatments for syphilis.**
  - **Patients were smeared with a cream containing a high concentration of mercury.**
    - **shut into a small hut called a 'stew.'**
    - **many died from the treatment**
    - **few were cured.**
  - **Ingestion of arsenic derivatives introduced in the 1800s.**
    - **many deleterious side effects that did not cure disease**
    - **in 1909 Paul Ehrlich developed an arsenical derivative (salvarsan), that was effective in reducing severity.**
      - **years of treatment**
      - **toxic**
      - **remained the treatment until 1943**

Tuskegee Syphilis Study

- **1932-U.S. Public Health Service began a study of the disease and enlisted 399 poor, black sharecroppers living in Macon County, AL., all with latent syphilis.**
- **No informed consent.**
- **The men or their families were not told they had syphilis but were told they had 'bad blood.'**
- **Cooperation was obtained by:**
  - **offering financial incentives (free burial if they agreed to an autopsy)**
  - **free physical exams**
  - **medications such as 'spring tonics' and aspirin**

Tuskegee Syphilis Study

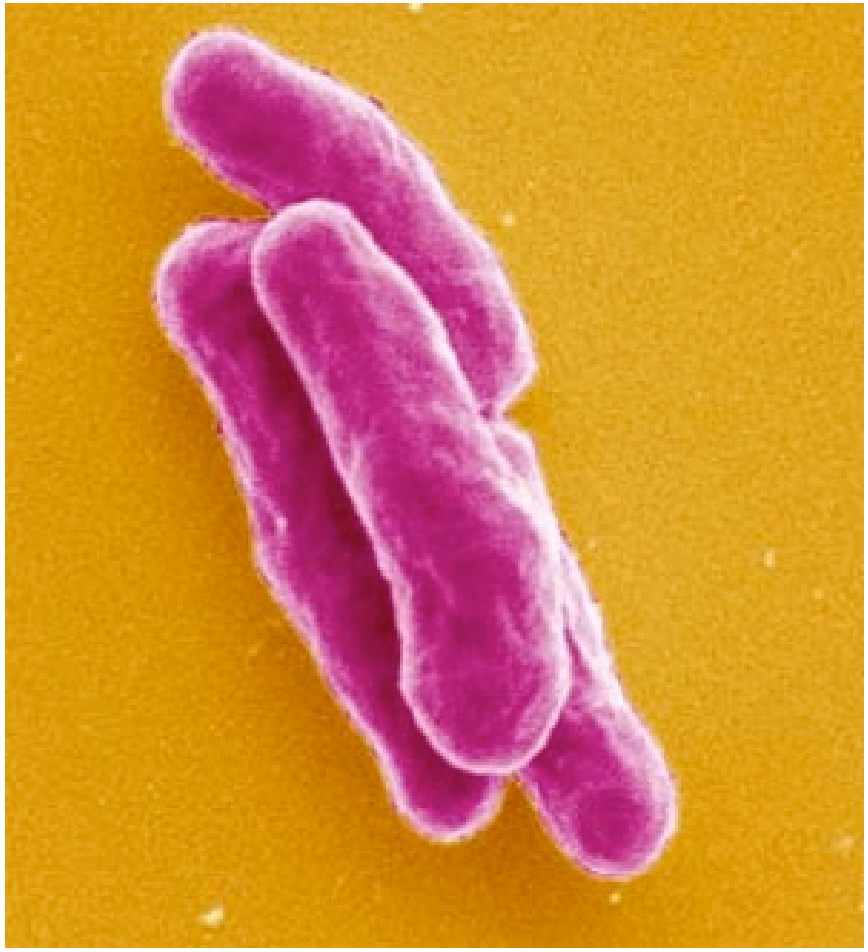
- **The men enrolled in the study were denied antibiotics even after penicillin came into use in 1947.**
- **By the time the study was made public, largely through James Jones' book *Bad Blood* and the play *Miss Evers' Boys*,**
  - **28 men had died of syphilis**
  - **100 other dead of related complications**
  - **at least 40 wives had been infected**
  - **19 children had contracted the disease after birth**

Tuskegee Syphilis Study

- **The study was 'designed' to document the natural history of syphilis.**
  - **It came to symbolize, however, racism in medicine.**
  - **Ethical misconduct.**
  - **Government abuse of society's most vulnerable--the poor and uneducated.**

Mycobacterium tuberculosis-- **The People's Plague**

- **Gram +**
- **Aerobic rod**
- **Lipid-rich cell wall**
  - **resistant to detergents**
  - **antigenicity**
  - **resistance to antibiotics**



Tuberculosis before antibiotics

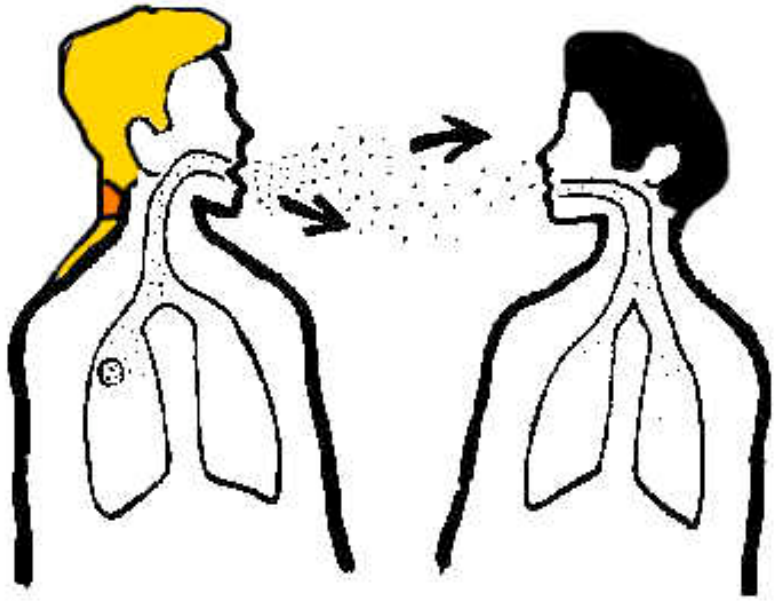
- **Although much feared, it was considered by some to be a 'romantic disease.'**
  - **Many artists and writers contracted it.**
- **It caused an ethereal pallor in the sufferer, highlighted by a rough-like coloration of the cheeks.**
  - **Never mind that the breath of tuberculosis sufferers was foul enough to put off the most devoted admirer.**

Tuberculosis Before Antibiotics

- **What was happening inside the patient was the very opposite of romantic.**
- **Once inhaled into the lung, the bacteria begin to multiply.**
- **The 5% of people that can not bring the infection under control, the bacteria cause so much damage to he lungs that the patient coughs up blood and eventually dies.**
- **In some people the bacteria move into the blood and disseminate to other organs, causing an even more aggressive and rapidly fatal disease known as 'galloping consumption.'**

Different from Syphilis...

- **In the case of syphilis, a person could avoid contracting the disease by living a monogamous lifestyle...**
- **Tuberculosis is contracted by inhalation.**



Tuberculosis Before Antibiotics

- **Tuberculosis patients who could afford the most advanced medical treatments of the time went to sanitaria in the mountains, where they spent hours in the open air and ate rich meals in an attempt to counter the wasting of the body.**
- **Pleasant as this may seem to today's spa enthusiasts, it was sadly unsuccessful.**

Excerpt from Thomas Mann's *The Magic Mountain* (Nobel Prize winning German novelist)

At first, Hans is delighted with his room, but Joachim nonchalantly exposes the rooms dark history. "'An American woman died here day before yesterday,' said Joachim. 'Behrens [a sanitarium administrator] told me directly that she would be out before you came...Night before last, she had two first-class hemorrhages, and that was the finish. But she has been gone since yesterday morning, and after they took her away of course they fumigated the room thoroughly with formalin, which is the proper thing to use in such cases.'"

Although formalin (a general poison that kills bacteria as well as people) may have prevented acquisition of the bacteria from inanimate objects but did nothing to prevent transmission from respiratory droplets from other patients.

Preventive strategies...

- **Devised by rural farmers in the Northeastern U.S. in the 1700s.**
- **Reveals the desperation that people of the time felt when they or members of their family faced the dreaded disease.**
- **This particular 'treatment' was based on the theory that tuberculosis was caused by vampires.**
  - **The Therapy: disinter the recently diseased and rearrange their bones to make it difficult for them to rise from their graves and walk among the living.**

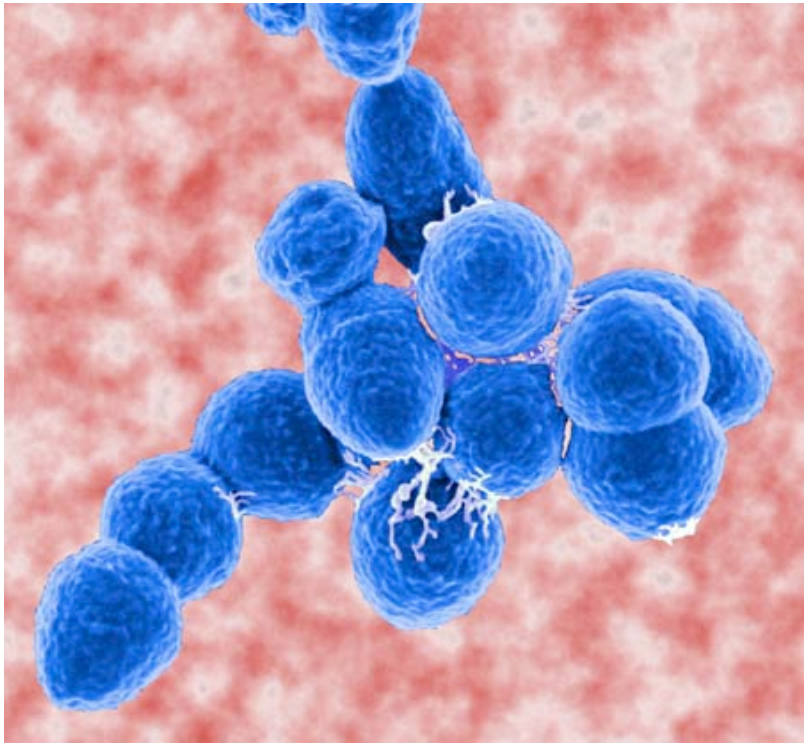
Antibiotic Treatment for TB

- **Therapy for at least 6 months.**
- **It still a scourge worldwide**
  - **Many countries have let down their guard.**
  - **TB has been particularly tragic in places like Africa, with its high rate of HIV infection.**
  - **Some have called TB and HIV the 'one-two' punch of death.**
  - **Unlike syphilis, TB is not likely to be eradicated soon.**

  - **1/3 of the worlds population currently infected with *M. tuberculosis*.**

Streptococcus pneumoniae

- **Gram + cocci**
- **Bacterial pneumonia**
- **The leading cause of infectious disease deaths in the U.S., more common than HIV.**
- **Where is the news regarding a disease that kills tens of thousands of people a year?**

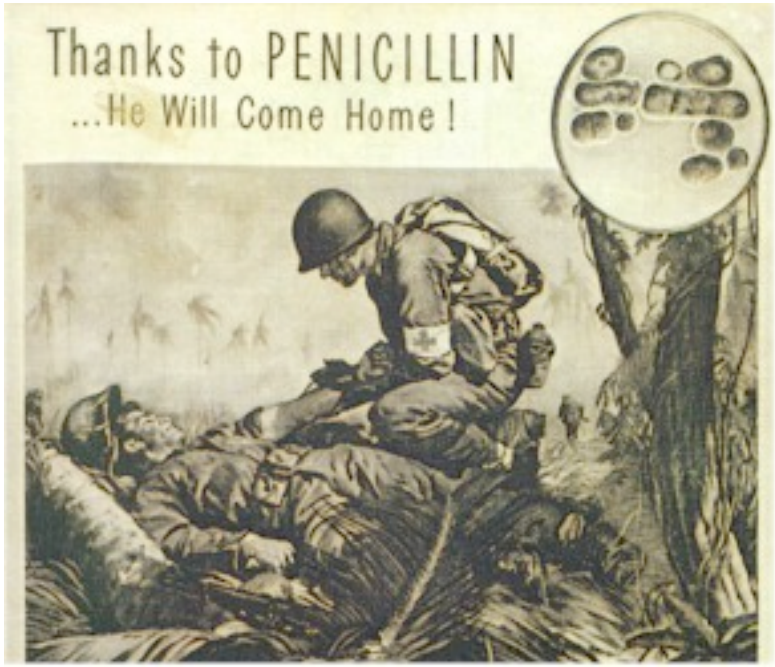


New York Times obituary in 1999.

- **Anne Sheafe Miller died at the age of 90, after a long, rich life.**
- **She was the first U.S. citizen whose life was saved by penicillin.**
- **“News of Mrs. Miller’s full and miraculous recovery inspired the pharmaceutical industry to begin full-scale production of penicillin.”**

Thanks to penicillin...

- **World War II was the first war in which wound infections were not the major cause of amputations and death.**

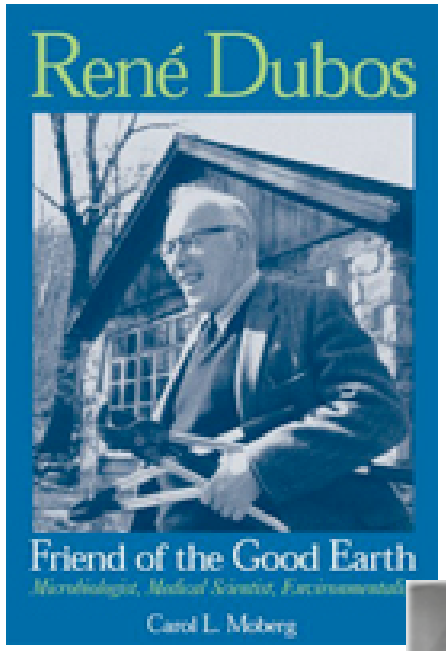


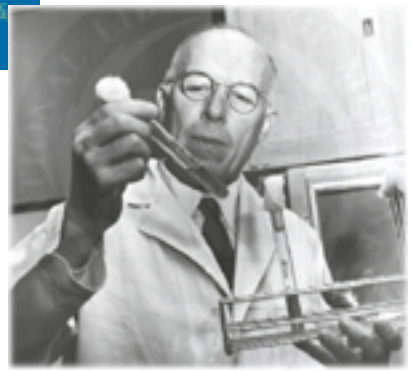
A Brief Look at the History of Antibiotics

- **The early approach to therapy was based on what has been called the poison principle.**
- **That is, known poisons were administered in limited doses in the hope that the bacteria causing the infection would be killed before the patient.**

René Dubos--Rockefeller University

- **Different view of how antimicrobials should work.**
- **Had an almost religious reverence for the purifying properties of soil.**
- **Mixed a culture of *S. pneumoniae* with an aqueous extract from soil.**





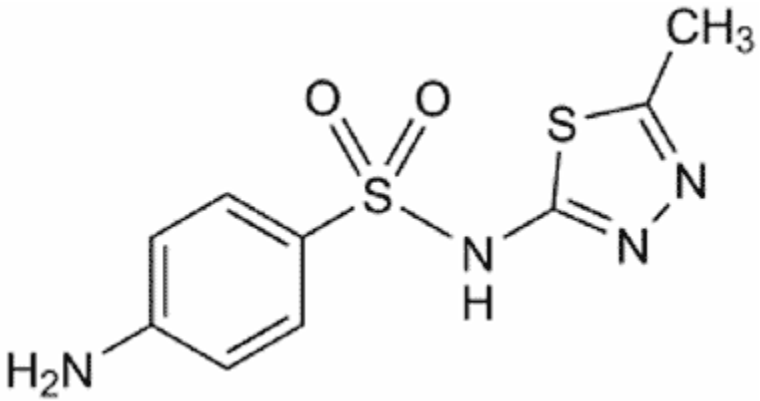
Dubos' theory was that there must be bacteria in soil that could kill or inhibit the growth of *S. pneumoniae* because the ecological balance would be maintained only if such microorganisms existed.

Dubos' Isolation of Soil Bacteria

- **Isolated a soil bacterium, *Bacillus brevis*, which produced a substance that was antagonistic to the growth of *S. pneumoniae*.**
- **The antibacterial compound isolated was a 'crude brownish material that...congealed into a sticky mass as unpleasant as so much uncouth earwax. But it was powerful all right.'**
  - ***Launching the Antibiotic Era*--Rollin Hotchkiss**
- **The active compound was gramicidin; a pore forming peptide. What else forms pores?**
- **Too toxic for internal use but still used as an ingredient in topical antibacterial preparations.**

The Sulfa Drugs

- **Resulted from a quest parallel to Dubos' search.**
- **This approach was to modify compounds that kill bacteria to make these compounds <u>less toxic</u> for humans.**
- **Synthetic compounds called sulfonamides were the result.**



How Sulfa Drugs Work

- **Mimic para-aminobenzoic acid, a precursor of the vitamin folic acid.**
- **Bacteria have to make their own; humans do not.**
- **Because of this, chemical mimics of para-aminobenzoic acid affected bacteria adversely by inactivating an enzyme the bacteria needed to make folic acid.**
- **Selective toxicity.**

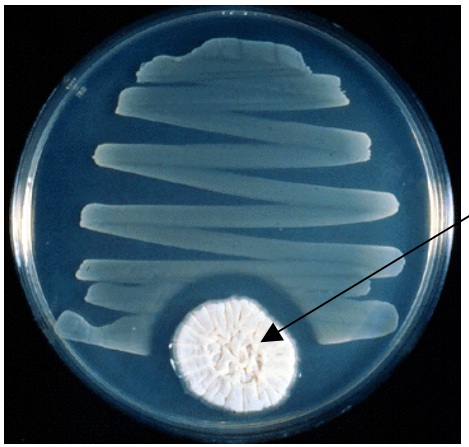
Penicillin is Discovered (by accident)

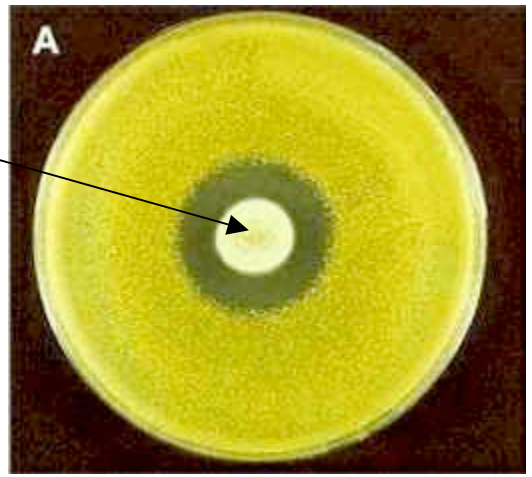
- **Alexander Fleming is credited for the discovery of penicillin when while he was working on treatments for wound infections caused by *S. aureus*.**
- **He was working with an arsenic derivative (arsphenamine) because of its success in curing some cases of syphilis.**
- **Fleming discarded agar plates containing colonies of *S. aureus* into trays filled with disinfectant...**

Excerpt from *Launching the Antibiotic Era*

Section written by Norman Heatley, one of the early workers in the area of antibiotic research.

Penicillium notatum





Unsung Heroes

- **Australian scientists Howard Florey and Ernst Chain.**
- **Did not see penicillin as another microbiological curiosity, as Fleming did initially, but instead dedicated themselves to discovering how to produce enough penicillin to make the drug widely available.**

Problems from the beginning...

- **Large cultures of *P. notatum* grown to harvest penicillin had very low activity.**
- **Chain realized that the low potency of the culture fluid was due to the fact that they were contaminated with *E. coli*--a strain that produced an enzyme that degraded penicillin.**
- **First (and immediate) glimpse that bacteria could become resistant to penicillin...**

Bacteria Reveal Their Adaptability

The rapidity with which some bacteria have developed mechanisms for resisting antibiotics should not have come as a surprise.

Why the Appearance of Bacteria Resistant to Antibiotics Was a Forgone Conclusion

- One of the first forms of life.
- Ruled the earth for over 3 billion years.
- Colonized the deepest parts of the ocean.
- Highest mountains.
- Artic and Antarctic.
- Nuclear wastes.
- As far underground in the land masses as humans have dug.
- Found in clouds.
- Inside rocks.
- Survived the volcanic era, temperature ranges.
- Boiling springs.

Is it ANY wonder that antibiotics represented at best a minor nuisance in the long march of their evolution?

Is it possible that we are in a race with bacteria that we will ultimately lose? Could we return to a preantibiotic era?

- **2000 B.C.--Here, eat this root.**
- **1000 A.D.--That root is heathen. Say this prayer.**
- **1850 A.D.--That prayer is superstition. Here, drink this potion.**
- **1920 A.D.--That potion is snake oil. Here swallow this pill.**
- **1945 A.D.--That pill is ineffective. Here, take this penicillin.**
- **1955 A.D.--Opps...bugs mutated. Here take this tetracyline.**
- **1960-1999 A.D. 39 more 'oops'...here, take this more powerful antibiotic.**
- **2000 A.D.--The bugs have won! Here, eat this root...**

Strategies for Becoming Antibiotic Resistant

- **1-destroy the antibiotic**
- **2-do not let the antibiotic reach toxic levels**
  - **protein pumps that keep concentrations low.**
- **3-chemically modify the target or mutate the target of the antibiotic.**
- **4-plasmid acquisition.**

Is Resistance Always the Bacteria's Fault?

- Treatment failure for other reasons than resistance to antibiotics:
  - Misdiagnosis of infection.
    - *C. albicans*-UTI
    - Protozoa or fungi--genital or lung infections
  - Use of antibiotic with the wrong pharmacokinetic properties.
    - tissue penetration
  - Failure to take the full course of the antibiotic.

****Rapid diagnostics could help make sure antibiotics are used prudently.**

Origins of Bacterial Resistance Genes

- Resistance genes usually resemble 'house-keeping genes.'
- Germ warfare?
  - no antibiotics in soil.
  - no pure cultures.
- Signaling molecules?
  - some bacteria that do not make antibiotics have resistance genes.
  - are these gene products receptors?

Bottom line: **Many antibiotic resistance genes were present long before humans began to produce them.**

Antibiotic Resistance in the News

Reporters Discover a New Scare Story

- **Prior to the 1990s antibiotic-resistant bacteria were rarely seen in the media.**
- **In the 90s, however, scare stories on the subject were common *e.g.*, headlines that read:**
  - **'Return to the Pre-antibiotic Era'**
  - **'Superbugs on the march'**
    - Basically, these stories supported/support the notion that we are headed back to pre-antibiotic times.

A More Realistic View

- **MANY disease-causing bacteria *e.g.*, *S. pyogenes*, *C. trachomatis* (gonorrhea-like infection), and *T. pallidum* have remained susceptible to most antibiotics.**
- **In addition, we have learned much about about preventing infections:**
  - **disinfectants**
  - **latex gloves**
  - **improved nutrition**
  - **smarter anti-cancer drugs**
  - **more rapid operations + less invasive**
  - **vaccines**

9/11 and Anthrax

- **Congressional workers got ciprofloxacin (60 day regimen) and postal workers got doxycycline.**
- **Many antibiotics besides Cipro are effective against anthrax. One is doxycycline, which is cheap and has few side effects.**
- **Class warfare issue? You bet!!**
- **Cipro was technically the only FDA-approved drug to treat anthrax.**

The Cartoon Equivalent of the FDA

- There are many cases in which the FDA has not approved a drug for a certain application because it was ineffective or dangerous, **BUT in the case of doxycycline**, economics and timing of approval were responsible for the "approved" status of Cipro and the "not approved" status for doxycycline.

Why?

- **Doxycycline, although a better drug for the treatment of anthrax was not listed as as a treatment for this disease because when doxycycline cam up for FDA approval, no one in their right mind would have listed anthrax as a disease treatable by the antibiotic.**
  - **This was post the Gulf War!**
- **Patent protection...**

The Irony of it All...

- **Is that the supposedly favored congressional workers got a drug that was hideously expensive ($700 for the course/person) and had unpleasant side effects, whereas the postal workers got an equally effective but much cheaper antibiotic with few side effects!**

‘How are you going to keep them on the farm after they’ve seen antibiotics?’

- **By June 2002 two bills before Congress that would limit antibiotic use in agriculture.**
  - **Brown bill in the House**
  - **Kennedy bill in the Senate.**
- **In June of 2003, a force far more powerful than Congress in the area of agricultural use of antibiotics entered on the side of limiting the use of antibiotics as growth promoters in animal husbandry...**

‘How are you going to keep them on the farm after they’ve seen antibiotics?’

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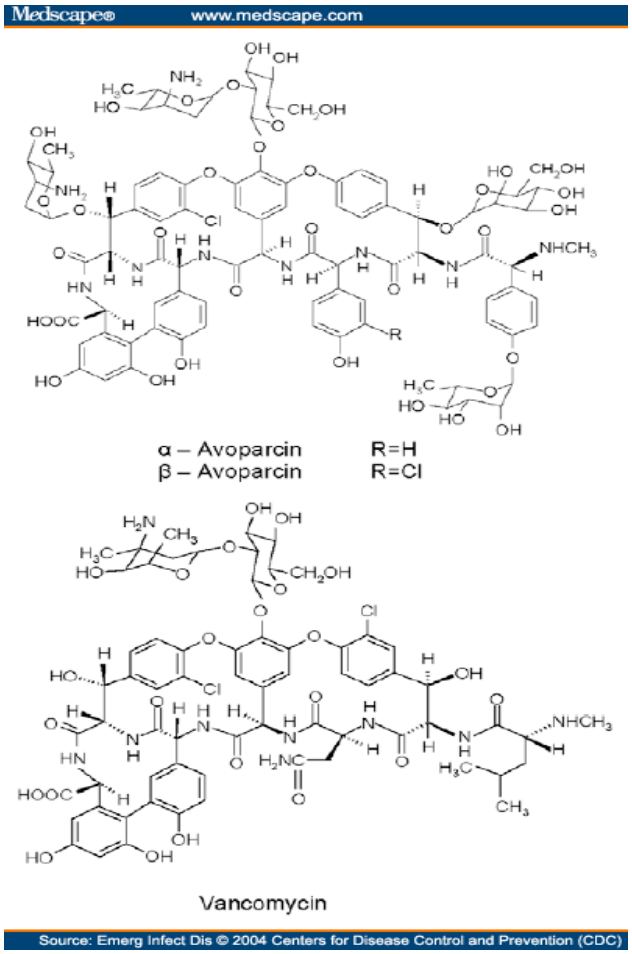
  - **McDonalds.**
    - **The public and the medical community may not listen to research scientists, but when McDonalds speaks...**

Agricultural Use of Antibiotics??

- **Basically, it boils down to the fact that as many TONS of antibiotics are used in agriculture as there are used to treat human disease.**
  - **complacency--it was believed that the antibiotics used in agriculture were different than those used for humans...oops!**
    - **used to treat sick animals (10-15% of use).**
    - **prophylaxis (30% of use).**
    - **Growth promoters (50-55% of use).**

Are 'Agricultural' Antibiotics that Different?

- **Avoparcin is a structural analog of vancomycin, one of the last-ditch human use antibiotics.**
- **Avoparcin can select for bacteria that are also resistant to vancomycin.**

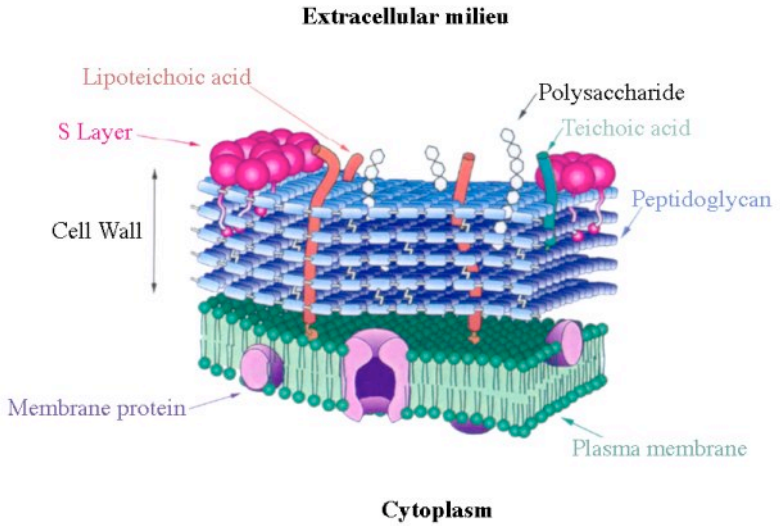


They're Everywhere!

- **In 1998, German scientists were measuring concentrations of various chemicals in the water just released from a sewage treatment plant.**
- **Identified high levels of fluoroquinolone antibiotics.**
  - **They don't go away when they get flushed!**
    - **They can enter water supplies and soil.**

Antibiotics that Inhibit Bacterial Cell Wall Synthesis

- **Penicillin and <u>related</u> drugs.**
  - **ampicillin**
  - **amoxicillin**
  - **vacnomycin**
  - **fosfomycin**
  - **bacitracin**



General Mechanisms

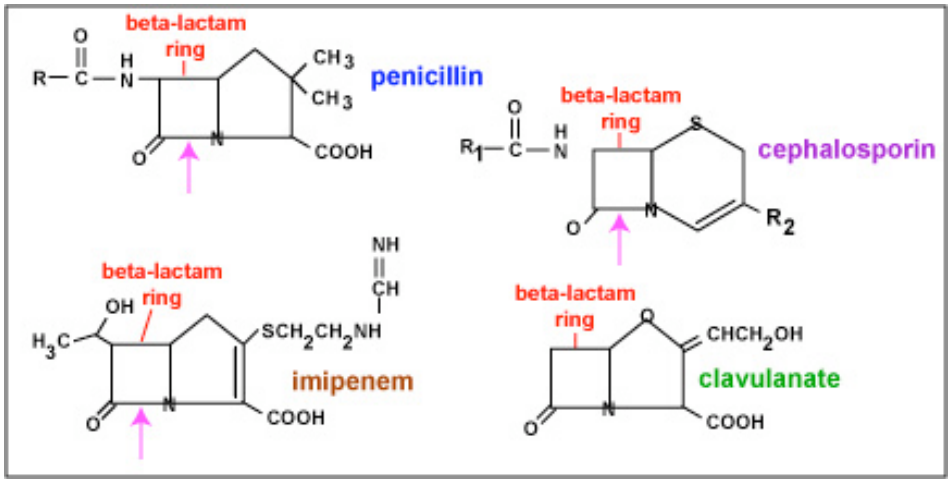
- **Fosfomycin-prevents peptidoglycan subunit synthesis (has to enter cell).**
- **Bacitracin-interferes with its export by interfering with bactoprenol.**
- **Penicillin/vancomycin have to get to the cell membrane (diffuse through the Gram+ cell wall) to interfere with peptide bond synthesis.**

Gram+ vs. Gram-

- **Gram negative cell wall impervious to the diffusion of most molecules through the outer membrane.**
- **Porins.**
- **Porin openings prevent some large antibiotics from passing through the outer membrane, making Gram- bacteria resistant to certain antibiotics.**
- **Also, some bacteria can modify the pore openings (make them smaller).**

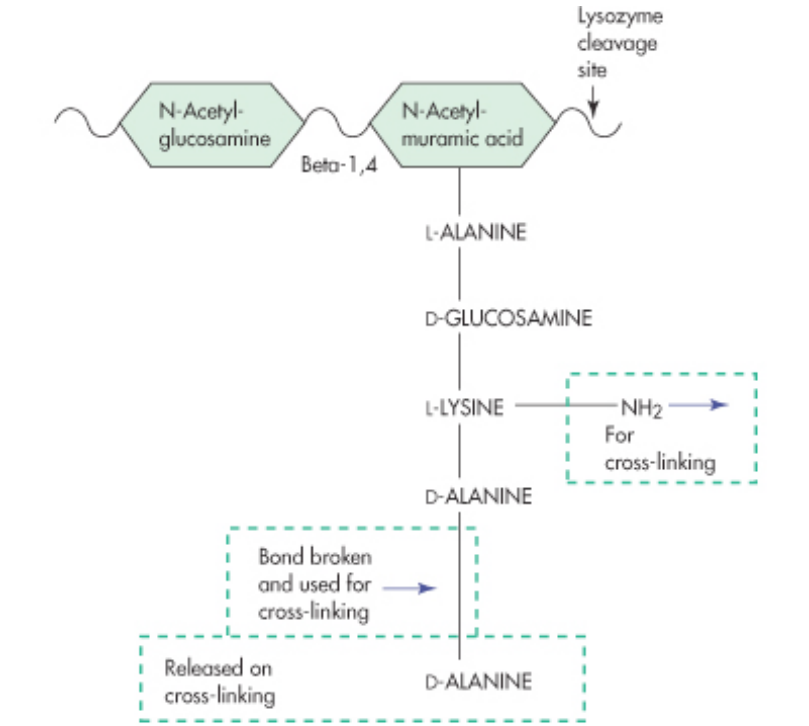
Penicillins and Cephalosporins

- **Related--have common β-lactam ring.**
- **Consists of 3 carbons and one nitrogen.**
- **Active portion.**
- **New additions:**
  - **carbapenems**
  - **monobactams**



Penicillin Mechanism

- **Prevents peptide cross linking.**
- **beta-lactam ring binds to these cross-linking enzymes (called penicllin-binding proteins or PBPs).**
- **beta-lactam similar to D-Ala-D-ala peptide and are thus attacked by cross-linking enzymes.**
- **Irreversible.**
- **Autolysins?**



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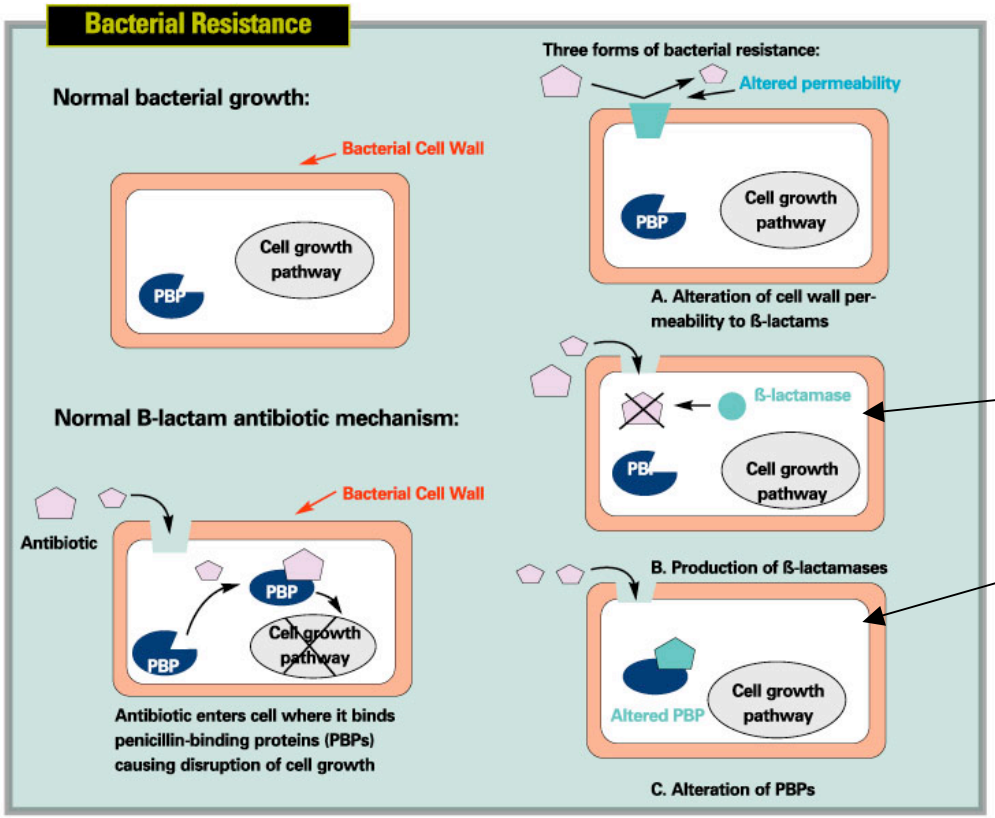
Resistance to Penicillin



Gram+ vs. Gram-

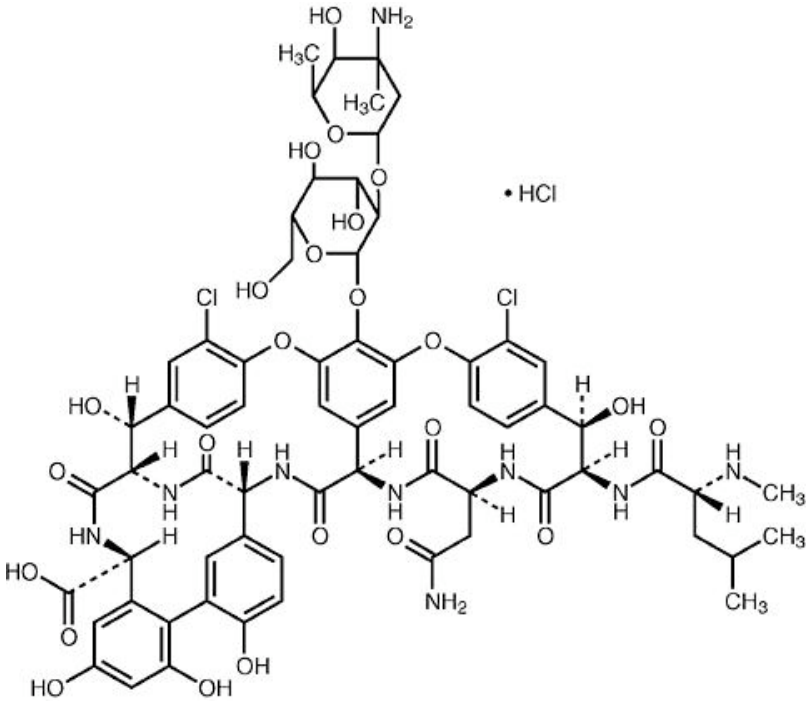
Diffusion vs. Guard dog.

Combating β-lactamase

- **Generate β-lactamse resistant antibiotics**
  - **Change the side groups surrounding the β-lactam ring.**
- **Develop antibiotic formulations containing both the antibiotic and a β-lactamase inhibitor.**
  - **amoxicillin + clavulanic acid=Augmentin**
- **This early success with β-lactamase inhibitors made scientists optimistic that they were winning the battle.**
- **Not long before bacteria changed their PBPs that no longer bound to β-lactams.**

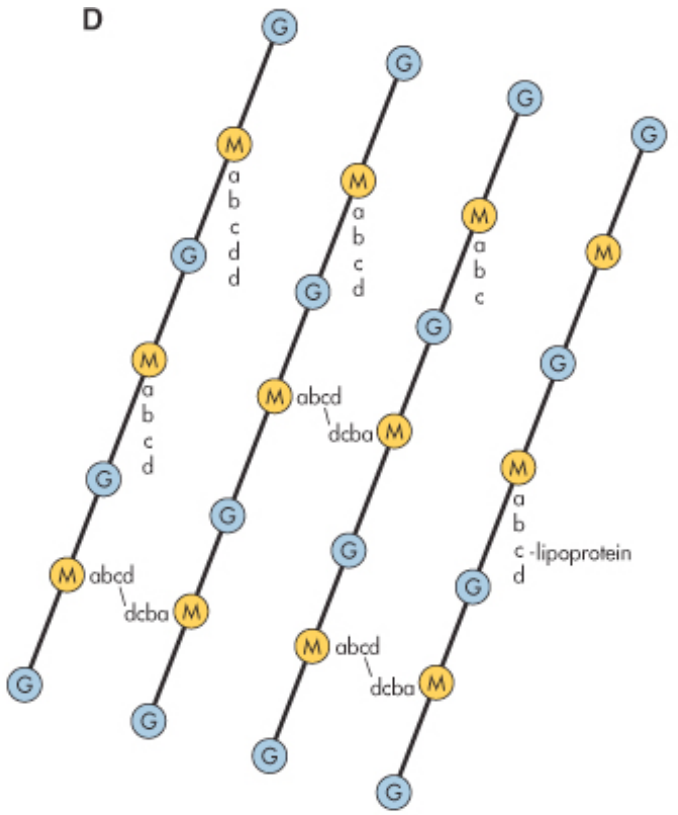
Rediscovering Vancomycin

- **Discovered in the 1960s.**
- **Several drawbacks:**
  - **Unappealing brown color.**
  - **Big. What does this affect?**
  - **Decrease in Gram + bacteria (at the time) and increase in Gram negative associated diseases from *e.g.*, *E. coli*, *Klebsiella sp.* and *Pseudomonas sp.***
  - **Now, Gram+ infections have rebounded.**
  - **Likely that the antibiotics used to treat Gram- bacteria opened the door for the Gram+ bacteria today.**



How Vancomycin Works

- **Like penicillin, it inhibits cross-linking.**
- **However, it binds to the peptides that are slated to become part of the cross-linked peptidoglycan structure.**



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Resistance to Vancomycin

- **How could a bacterium become resistant to such an antibiotic?**
  - **It would require the bacteria to change their cross-link peptide structure.**
    - **This might 'confuse' the cross-linking enzymes.**
  - **It would have to get rid of the old peptide.**
- **This is <u>exactly</u> what has happened.**
  - **unlike other resistant mechanisms, vancomycin resistance requires several genes.**
    - **Proteins that comprise a pathway for changing the peptidoglycan cross-linking peptides.**
    - **An enzyme to chop the D-Ala-D-Ala part of the original peptide**

Natural Resistance to Vancomycin in Some Bacteria?

- **Some Gram+ bacteria may not have a terminal D-Ala-D-Ala peptide.**
- ***Lactobacillus sp.*, used as probiotics (found in yogurt or consumed in powder form) that many or may not have a health benefit.**
- **Can hurt anyway. Right?**
- **Imagine the shock when it was demonstrated that most Lactobacillus strains being used as probiotics were vancomycin resistant.**
  - **Fortunately this is due to an innate trait in metabolism and can not be transmitted to other bacteria.**

Protein Synthesis Inhibitors

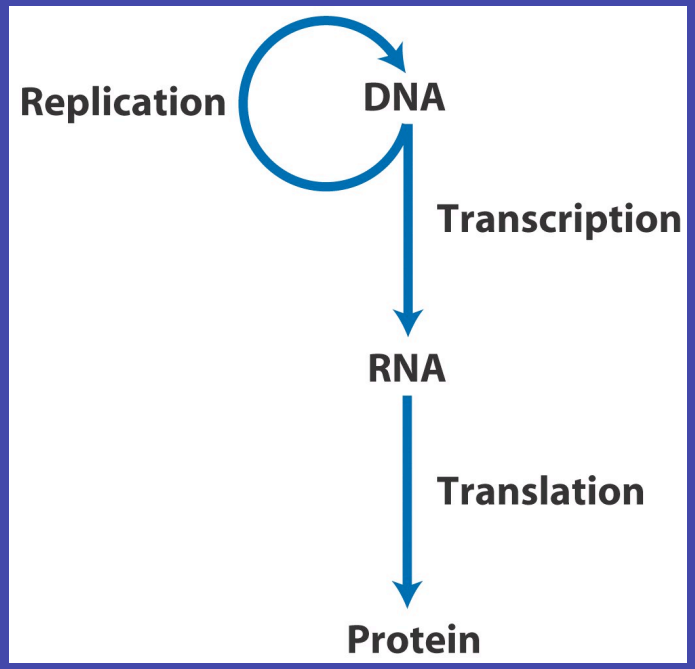
- **All of these antibiotics (with one exception) bind to bacterial ribosomes, the factories that translate the message in mRNA into proteins.**
- **Prevent bacteria from making proteins.**

Synthesis Inhibitors

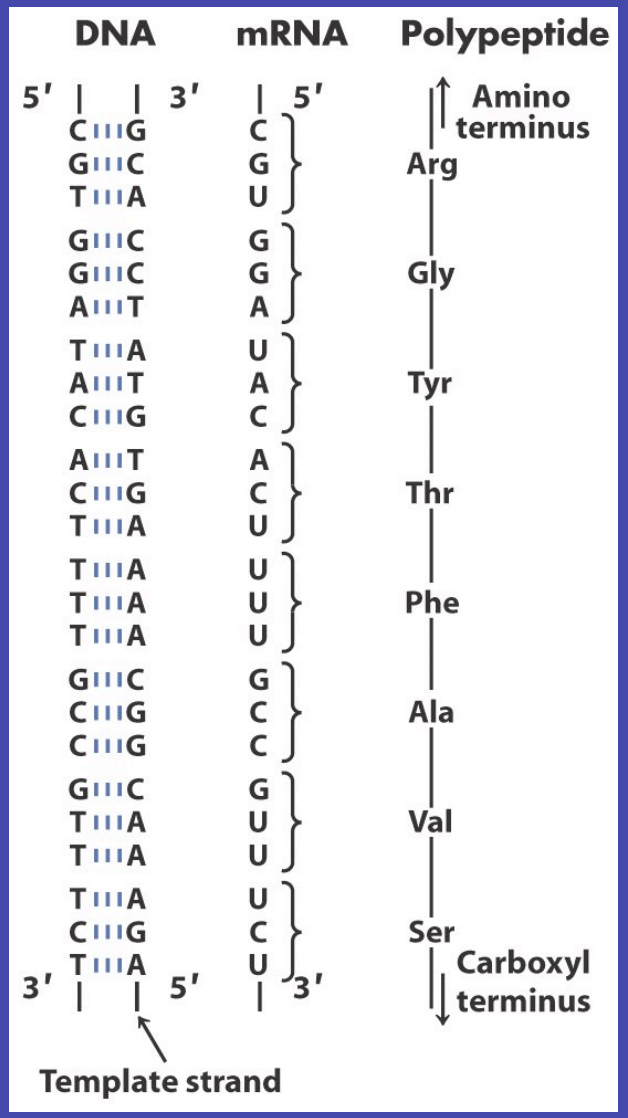
Family	Antibiotic	Brand Name
Aminoglycosides	Streptomycin Amikacin Neomycin	Steptomycin Amikin Neosporin
Tetracyclines	Doxycycline Oxytetracycline Minocycline	Monodox, Vibramycin Terramycin Minocin
Macrolides	Erythromycin Axithromycin	Erythrocin Zithromax
Lincosamides	Clindamycin	Cleocin
Streptogramin	Quinupristin + dalfopristin	Synercid
Oxazolidone	Linezolid	Zyvox
Mupirocin	Mupirocin	Bactoban

Utilization of genetic information

3 major processes:

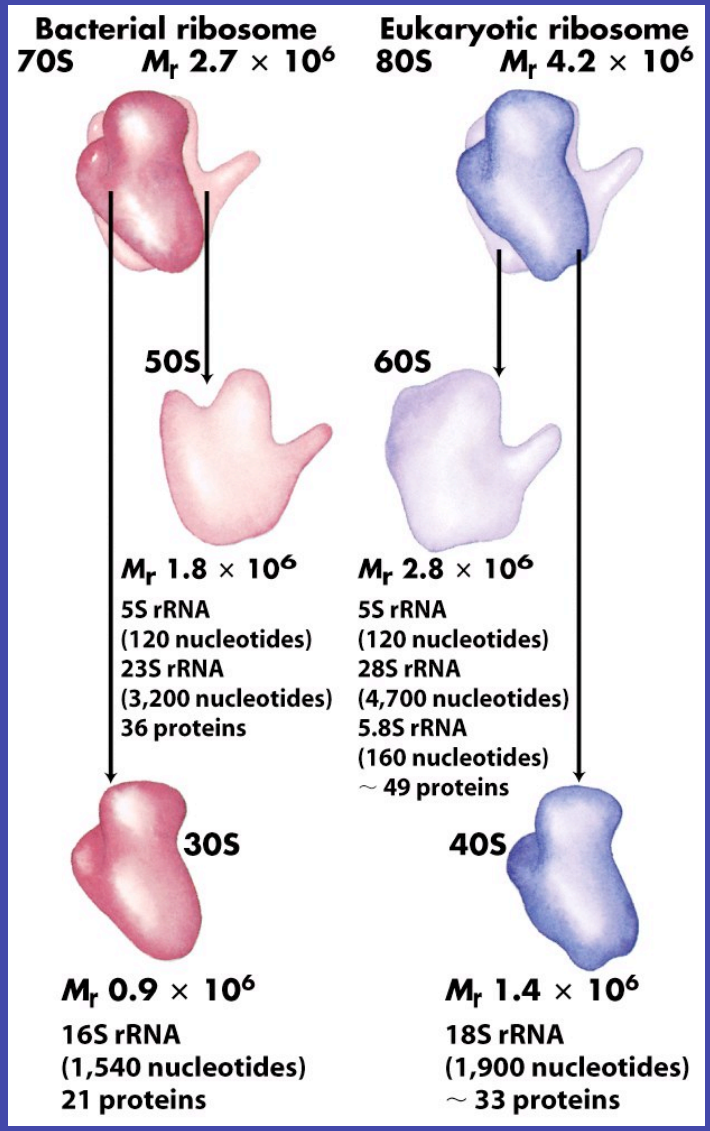


- Replication of DNA
- Transcription of mRNA from DNA
- Translation of nucleic acid sequence to amino acid sequence



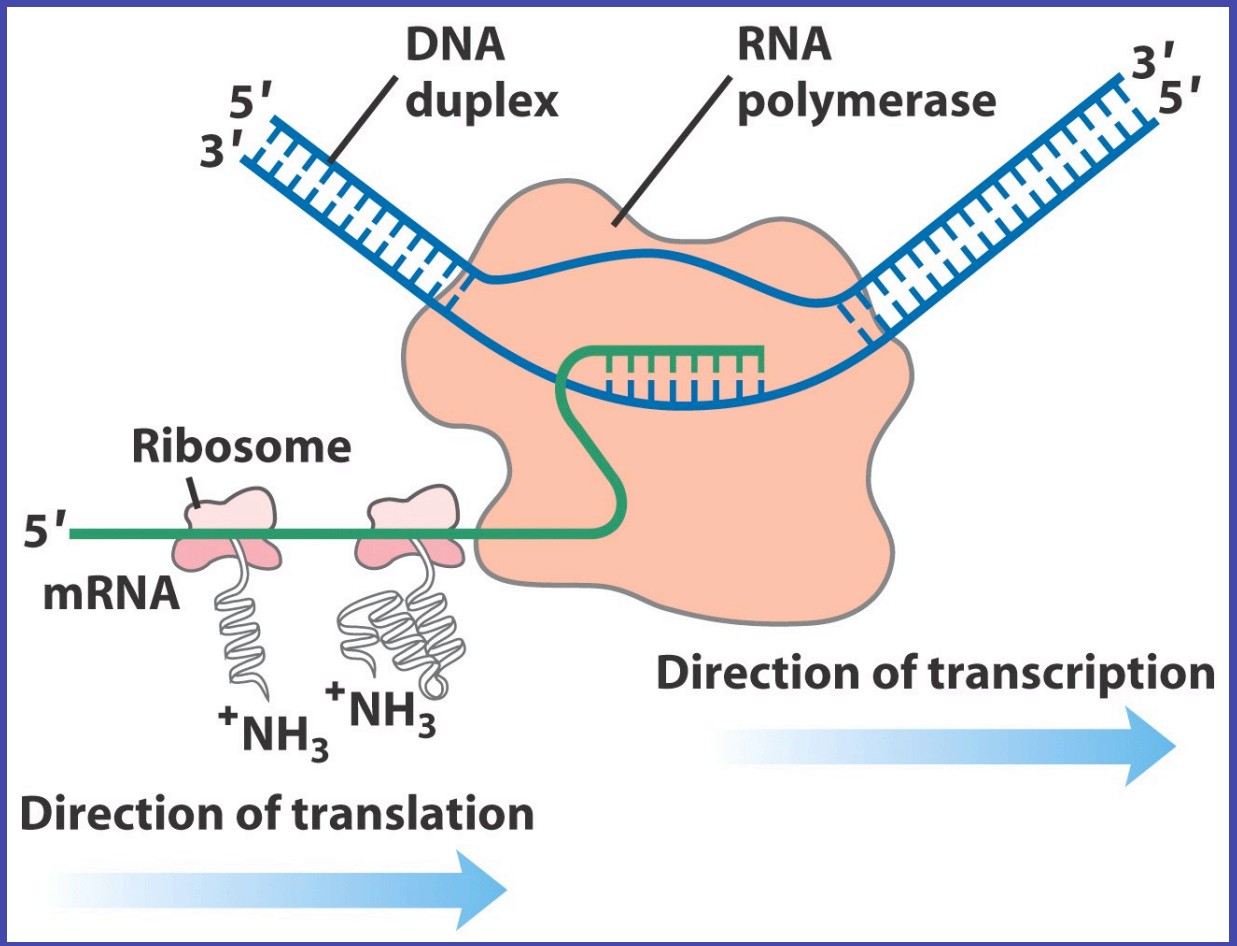
Ribosome composition

- Ribosomal subunits are named for their S (Svedberg unit) values, which are sedimentation coefficients indicating their rate of sedimentation in a centrifuge.
- Ribosomes consist of rRNAs (i.e. 5S and 23S) and many proteins.

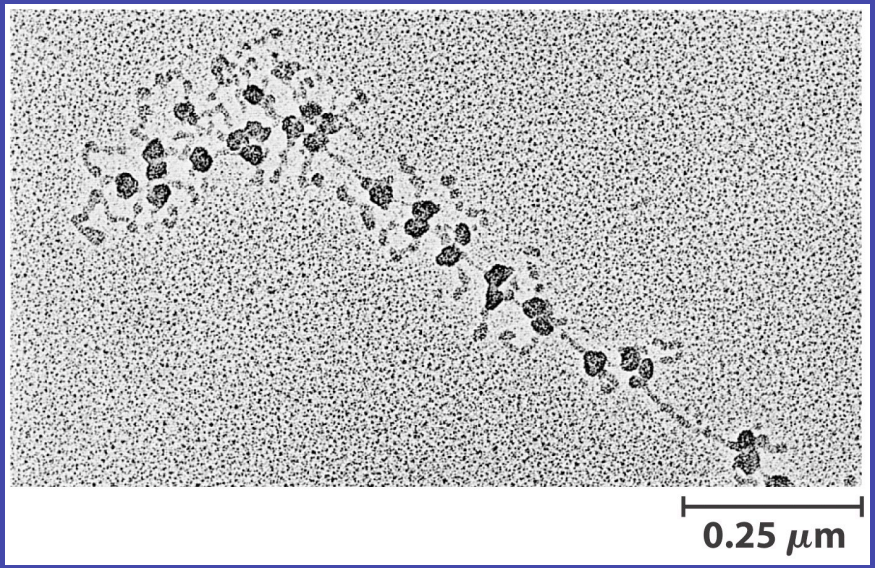


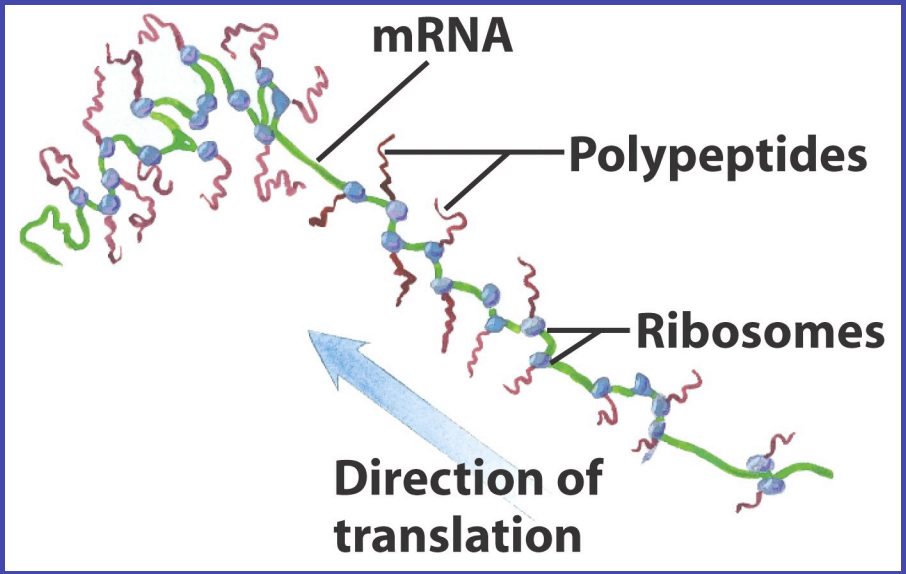
Transcription and translation

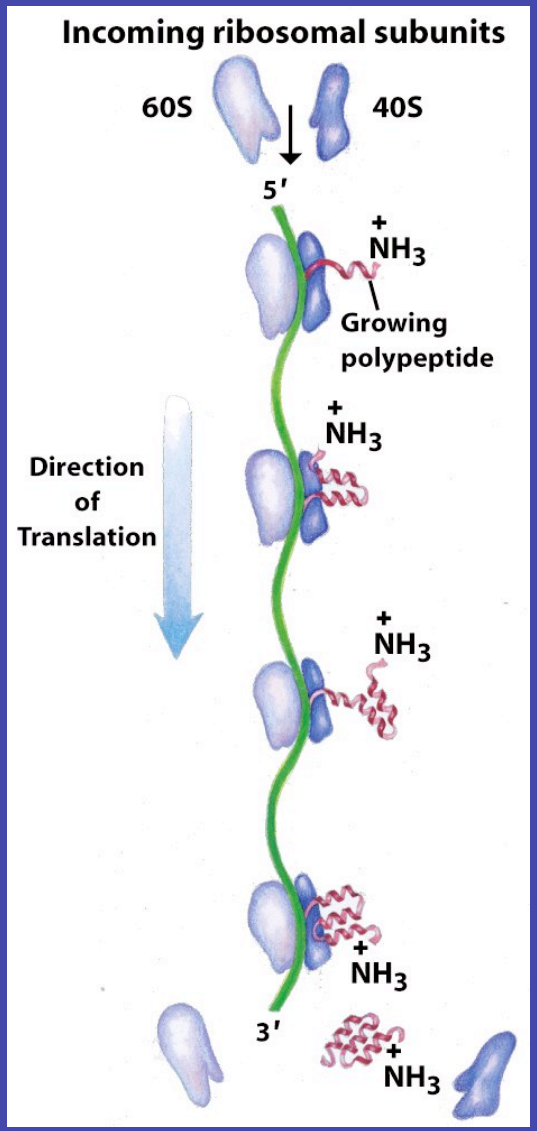
In bacteria, a coupled process is possible.



Polysome in eukaryote



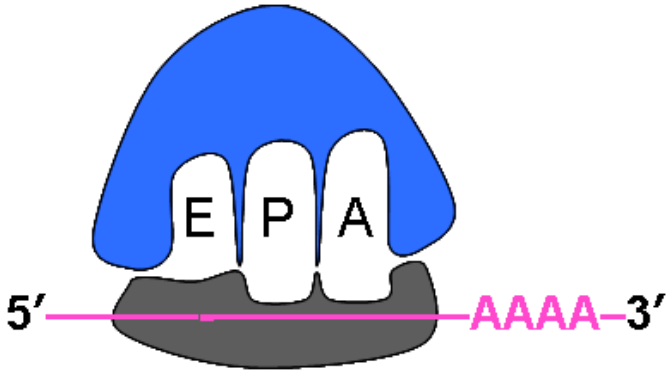




Protein Elongation

Ribosome Anatomy

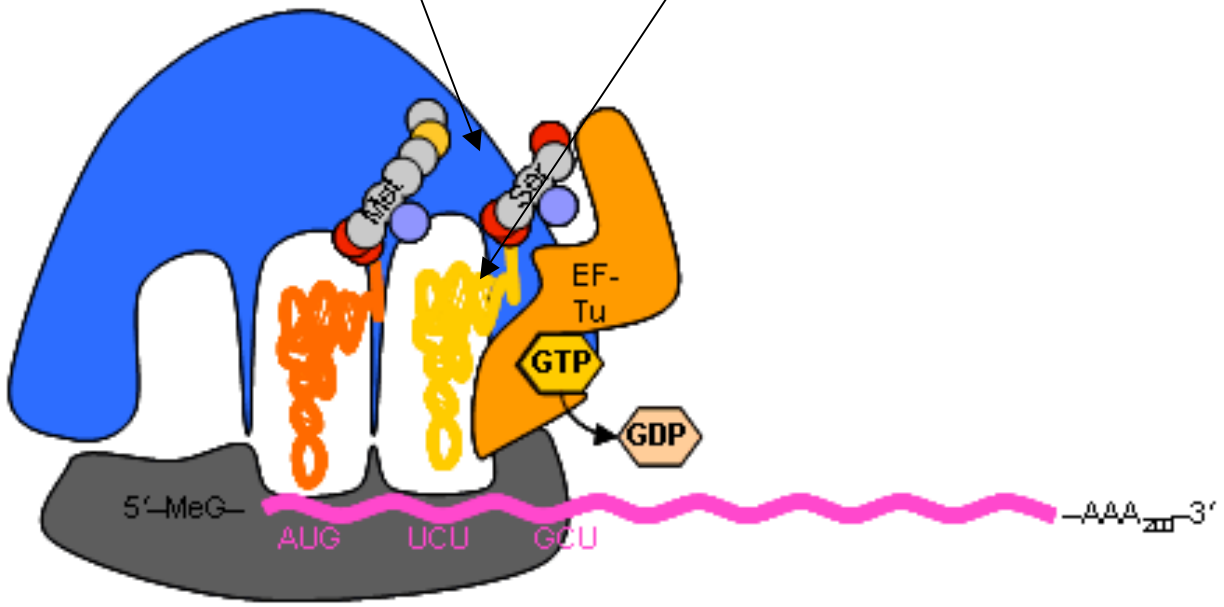
- **P site (peptidyl-tRNA site)**
- **A site (Acyl-tRNA site)**
- **E (exit)**



Antibiotic Interference

Erythromycin and lincosamides block elongation

Tetracycline distorts A site; tRNA cannot bind.



Linezolid blocks ribosome assembly.

Streptomycin and Other Aminoglycosides

- Streptomycin was one of the earliest antibiotics to enter the market.
  - Selman Waxman, Rutgers
    - Nobel Prize
- Binds the small RNA subunit and 'freezes' the ribosome on the mRNA.
- **Aminoglycosides named so because they are made up of sugars with amino groups attached to them.**
- Oddly enough, aminoglycoside other than streptomycin (*e.g.*, amikacin, kanamycin, neomycin) prevent later steps in protein synthesis despite their similar structures.

Administration of Aminoglycosides

- **Not readily absorbed from the intestine.**
- **Not given orally unless treating gut pathogens.**
- **Tolerated over the short term given i.v. or i.m.**
- **Exception is neomycin, sold over the counter.**
  - **to toxic for internal use...ointment**

Resistance to Aminoglycosides

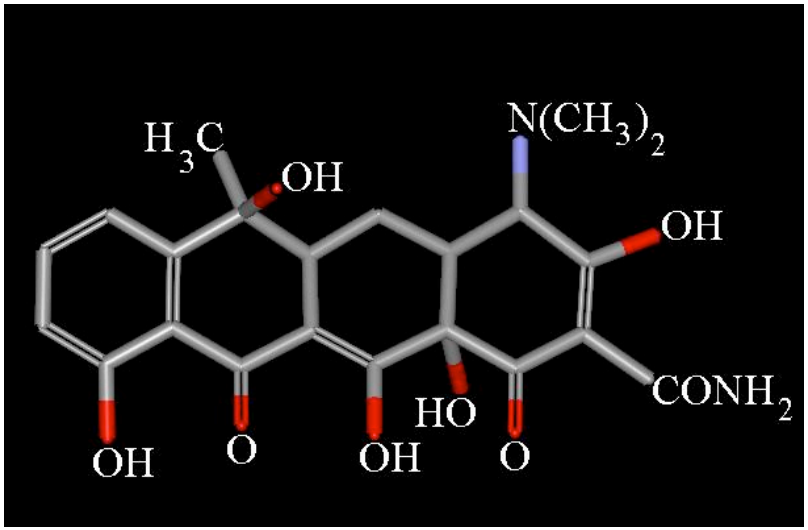
- **Mutation of ribosomal components**
  - **changes the target of the antibiotic**
- **Enzymes that modify aminoglycosides**
  - **prevents attachment to ribosome**
- **Fail to take them up**
  - **aminoglycosides are charged molecules that do not diffuse.**
  - **have to be pumped in**
  - **this type of resistance not well understood**

Aminoglycosides--Dangers

- **Toxic side effects**
  - **kidney damage**
  - **damage to the inner ear**
- **Initial stages reversible of antibiotic treatment is stopped.**
  - **continued use would result in permanent damage**

Tetracyclines

- **Characteristic four fused rings.**
- **Distorts the the structure of the small ribosomal subunit.**
- **Incoming tRNA molecules can't bind.**



Resistance to Tetracyclines

- **Tetracycline is not a charged molecule and can diffuse into the cell.**
  - **Efflux pumps**
- **Bacterial protein that protects against the ribosomal-modifying properties of the drug.**
  - **ribosomal protection-type tetracycline resistance.**
- **Chemical modification of tetracycline.**
  - this type of resistance only found in a group of bacteria that live in the intestinal tract but can also cause infections (*Bacteroides sp.)*.
  - The gene encoding this resistance in *Bacteroides sp*. can confer resistance to *E. coli*.

Other Tetracycline Notes

- **Yellowing of teeth in children.**
- **Over used as treatment for acne**
- **Many resistant strains.**
- **Still effective for Lyme disease and anthrax.**

Erythromycin & Other Macrolides

- **Successful in treating wound and respiratory infections.**
- **Binds large ribosomal subunit.**
- **Useful alternative for patients who are allergic to penicillin.**
- **Azithromycin (Zithromax) has been attracting attention as treatment for STIs.**
  - **easy to administer**
  - **expensive**

Clindamycin: Cures but also can Kill

- **Lincosamide**
  - **large subunit binder**
- **It was not until the 1980s that clinicians accepted that bacteria that could not divide in the presence of oxygen (obligate anaerobes) could cause disease in humans!**
  - **Most infections caused by *Bacteroides sp*.**
    - **usually after abdominal trauma**
    - **naturally resistant to many antibiotics**

Clindamycin: Cures but also can Kill

- **The problem with clindamycin and other antibiotics that are effective against obligate anaerobes is that they kill natural flora (that is protective).**
- **Opens the door for *Clostridium difficile* infections.**
  - **pseudomembranous colitis**

Fluoroquinolones, Sulfa Drugs, and Antituberculosis Drugs

Cipro and other fluoroquinolones are among the most frequently prescribed antibiotics today (in no small part because of aggressive marketing).

Fluroquinolones: Mechanism

- **Bind to DNA gyrase.**
  - **human gyrases different enough**
- **Mutations resulting in a gyrase not affected by the drug arise 1 in every 10 billion bacteria.**
  - **bacteria present at concentrations that are hundreds to thousands of times higher than this.**

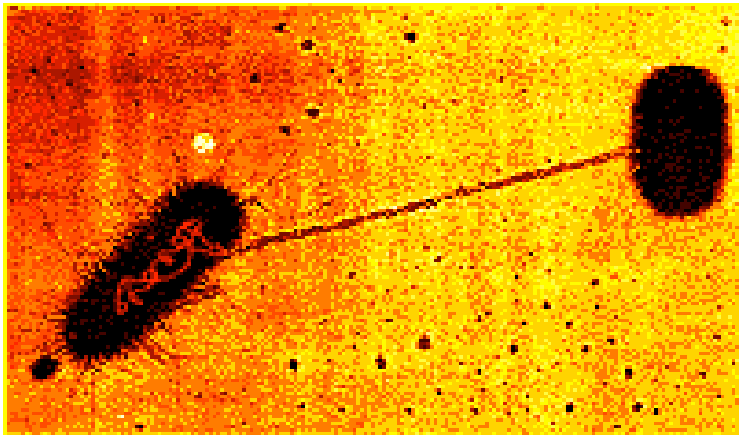
- **Enrofloxacin, *Salmonella enterica* and *Enterococcus sp.***

Bacterial Promiscuity: How Bacterial Sex Contributes to Development of Resistance

The Agony of Mutation, the Ecstasy of Getting the Mutated DNA from Someone Else

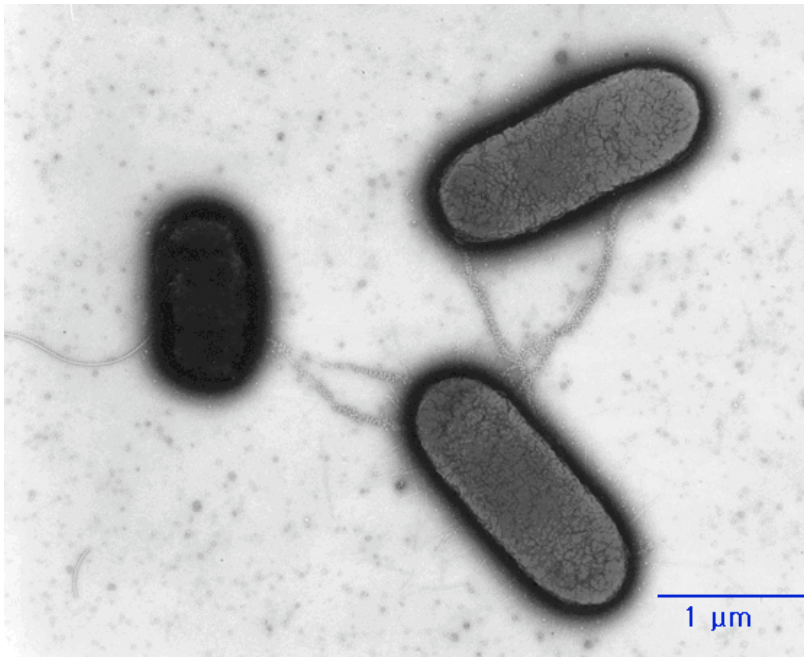
Achieving Resistance

- **Mutation (as discussed).**
  - **can be deleterious**
  - **can take time**
- **Acquire new DNA that confers antibiotic resistance.**
  - **1-from another bacteria**
    - **conjugation**
    - **F+ and F-**
    - **not all exchanges require a sex pilus**
  - **2-from the environment**
  - **3-phage**



Achieving Resistance

- **Bacteria can transfer DNA from one genus to another.**
- **Some bacteria can transfer DNA to plant cells, yeasts, and other eukaryotic cells.**
- **Can go three ways--triparental mating.**
  - **self-transmissible plasmid**
  - **mobilizable plasmid**



Resistance Gene Transfer and Agricultural Use of Antibiotics

- **Tons of antibiotics used in animal husbandry each year.**
- **Used to enhance growth (pigs and chickens).**
  - **speed of weight gain rather than larger animals**
- **Prophylaxis**
- **Tight profit margins.**

Europe vs. U.S. Farms

- **Europe**
  - **Farms spread out**
  - **Less density**
- **U.S.**
  - **farms confined to small areas**
    - **crowded living conditions**
    - **greater chance of infection**
  - **space for parks etc.**
  - **we want cheap meat!!**

-Antibiotic bacteria are selected in the intestines of antibiotic-fed animals.
-Bacteria colonize the carcasses
-Enter food supply...

The Fate of Bacteria...Once Consumed by Humans

- **Most bacteria should die if the food is well cooked (does not usually happen).**
- **Most bacteria eaten should then die in the stomach.**
- **Should not be able to compete with established flora.**
- **What they CAN do is transfer antibiotic resistance to the normal flora...only takes 24-48 hours.**

Use of Antibiotics in Human Medicine and Resistant Gene Transfer

- **In June 2002 a milestone was passed...**
  - **Vancomycin was the last line of defense against *Staphylococcus sp.* and *Streptococcus sp.***
- **The vancomycin barrier began to crack when *Enterococcus sp.* began to become vancomycin resistant.**
- **In 2003, the first fully resistant strain of *S. aureus* was reported.**
  - **this strain was isolated from a dialysis patient**
  - **such patients often develop mixed infections**
  - **in this case, both *S. aureus* and *E. faecium* were isolated from the patients blood.**