# Antibiotics

BIOTECHNOLOGY
AND USE

# What are antibiotics?

- Antibiotic (from the Ancient Greek: – anti, "against", and – bios, "life")
  - Substance produced by a microorganism
  - Kills or inhibits the growth of other microorganisms, namely bacteria
- Are antimicrobials (group includes anti-viral, anti-fungal, anti-parasitic drugs)
- Relatively harmless to the host, therefore can treat infection

# Classes of Antibiotics

- *Carbepenams*
- *Chloramphenicol*
- *2nd 3rd 4th Gen Cephalosporiins*
- *3rd Gen Fluoroquinolones*
- *Broad spectrum penicillins*
- *Tetracyclines*

- *Penicillin V and G*
- *Lincosamides(Clindamycin)*
- *Glycopeptides(Vanco and Teicoplanin)*
- *Streptogramins*
- *Rifamycins*
- *Daptomycin*

Broad Spectrum Coverage

Narrow Spectrum Coverage

- Broad-spectrum can act against a wide range of bacteria
  - Acts on both Gram-positive and Gram-negative bacteria
- Narrow-spectrum are only each effective against one specific family of bacteria

# Classes of Antibiotics: based on mode of Action

**EXAMPLES:**
Chloramphenicol
Erythromycin
Clindamycin
Sulfonamides
Trimethoprim
Tetracyclines


Bacteria are alive, but not capable of multiplication.

**Bacteriostatic**

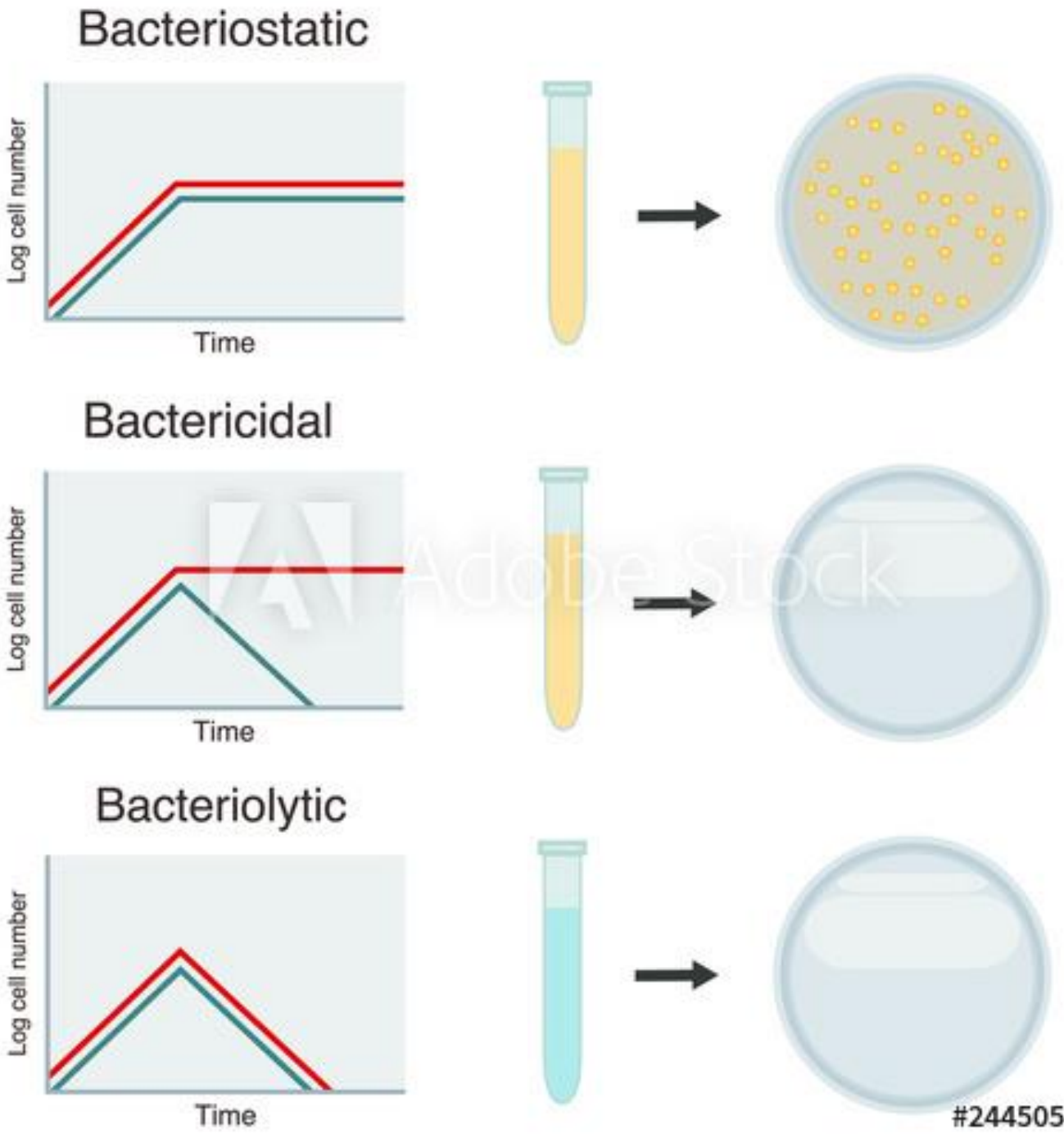

**EXAMPLES:**
Aminoglycosides
Beta-lactams
Vancomycin
Quinolones
Rifampin
Metronidazole

Bacteria die, but physically continue to be present in the environment.

**Bacteriocidal**

# Antagonism between Bacteriostatic and Bactericidal Antibiotics

- **Bacteriostatic antibiotics** limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism.
- **Bactericidal antibiotics** that inhibit cell wall synthesis: the *beta-lactam antibiotics* (penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems) and *vancomycin*. Also bactericidal are *daptomycin, metronidazole, nitrofurantoin, co-trimoxazole, telithromycin, fluoroquinolones*.
- **Bacteriolytic antibiotics** act to lyse the bacteria, dramatically decreasing the total cell count and the viable cell count. Many common antibiotics such as Penicillin act in this way. Bacteria die, bacterial cell walls are destroyed.


Bacteriostatic
Log cell number
Time
Bactericidal
Log cell number
Time
Bacteriolytic
Log cell number
Time
#244505415

# Major Classes of Antibiotics

- Aminoglycosides
- Beta-lactams:

  Penicillins

  Cephalosporins

  Carbapenems

  Monobactams
- Fluoroquinolones
- Glycopeptides
- Ketolides
- Lincosamides
- Macrolides
- Oxazolidinones
- Streptogramins
- Sulphonamides
- Tetracyclines

# History of Antibiotics

Rudolph Emmerich

## Late 1800s

- Germ theory accepted (bacteria as cause of ailments)
- The search for antibiotics began
- Scientists began to devote time to searching for drugs to kill the disease-causing bacteria
- German doctors, Rudolf Emmerich and Oscar Low
  - First to make an effective medication that they called pyocyanase from microbes
  - First antibiotic to be used in hospitals, however not often work

# History of Antibiotics

## 1928 and Penicillin

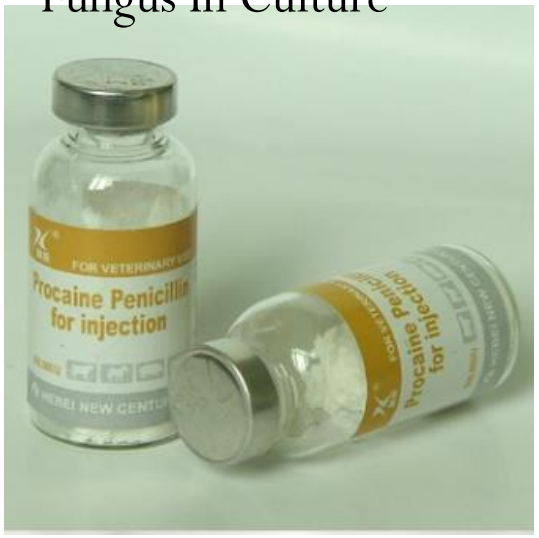
- The first effective antibiotic discovered
- French physician Ernest Duchesne noted in his 1896 thesis that certain Penicillium molds killed bacteria
- Sir Alexander Fleming's bacterium culture of Staphylococcus aureus was ruined by accidental fungal contamination
  - Fleming noticed a clear zone surrounding the colony of mold (Penicillium notatum)
  - Mold was secreting something that stopped bacterial growth
- Since the mold was of the genus Penicillium, he named this compound penicillin

**Ernest Duchesne** made this discovery 32 years before Alexander Flemming discovered the antibiotic properties of penicillin, a substance derived from those molds, but his research went unnoticed.

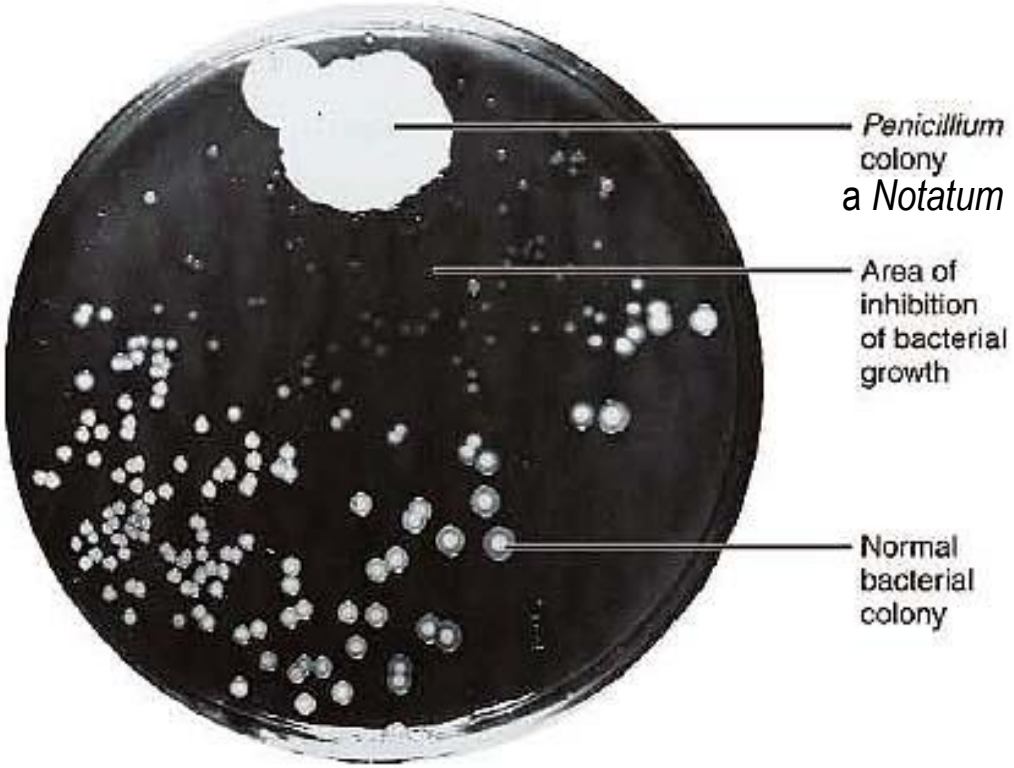
# History of Antibiotics

Sir Alexander Fleming,
discovered antibiotics in 1929.

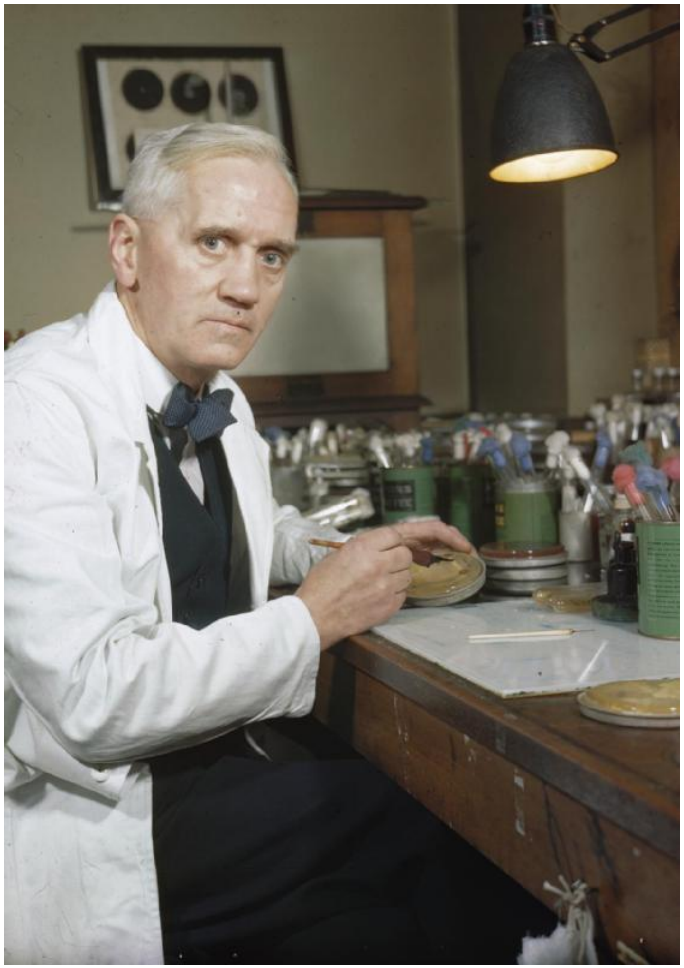
Penicillium Notatum
Fungus In Culture



# Produsing Antibiotics:




All instruments were unsuitable for large scale production, but in next years so far biotechnology has made great advances.



Sir Alexander Fleming, discovered antibiotics in 1929.

# History of Antibiotics

## The 1940s

- Manufacturing process for Penicillin G Procaine invented by Howard Florey and Ernst Chain
- Penicillin could now be sold as a drug
- American microbiologist Selman Waksman made streptomycin from soil bacteria
  - The first of a new class of drugs called aminoglycosides
  - Streptomycin could treat diseases like tuberculosis, but with severe side effects

# History of Antibiotics

## The 1950s

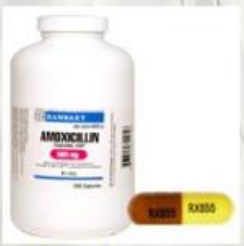
- 1955: Tetracycline patented by Lloyd Conover
  - Became the most prescribed broad spectrum antibiotic in the United States
- 1957: Nystatin patented and used to cure disfiguring and disabling fungal infections

Lloid Conover

# History of Antibiotics

SmithKline Beecham

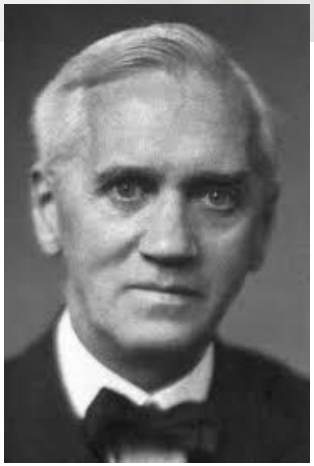
## 1981




- SmithKline Beecham patented Amoxicillin (amoxicillin/clavulanate potassium tablets)
- First sold the antibiotic in 1998 under the tradenames: Amoxicillin, Amoxil, and Trimox
    - Amoxicillin is a semi synthetic antibiotic

# Nobel Prizes

- 1939: Edward Chain and Howard Florey further studied penicillin
  - Later carried out human trials
- Fleming, Florey, and Chain shared the 1945 Nobel Prize for medicine for their work on penicillin
  - Ushered in the era of antibiotics



Alexander Fleming

Howard Florey

Adward Chain

# Why Microorganisms Produce Antibiotics ?

Even though antibiotics are entirely natural products, scientists have to admit that they are not quite sure why microorganisms produce them:

**Interspecific competition** - but this is only part of the entire story;

**Antibiotics are secondary metabolites** - once the microorganism runs out of nutrients, it is capable of breaking them down again to overcome a nutritional short supply

**Production of an antibiotic is a metabolic waste product**, besides being self-immune it keeps away competing organisms.

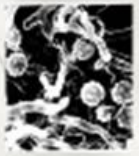
## Development with biotechnology

- Originally only derived from living organisms
  - Most new ones are semi-synthetic
  - Chloromycetin, cycloserine, and synthetic tetracycline were first produced completely by man
- Use new tools to culture on large scale (fermentation)
- New strains improve production and reduce costs
- Aim to make more specific to bacteria with fewer side effects

# Living sources

- Bacteria

  - Bacillus brevis
  - B. polymyxa
  - Steptococcus cremoris
- Actinomycetes



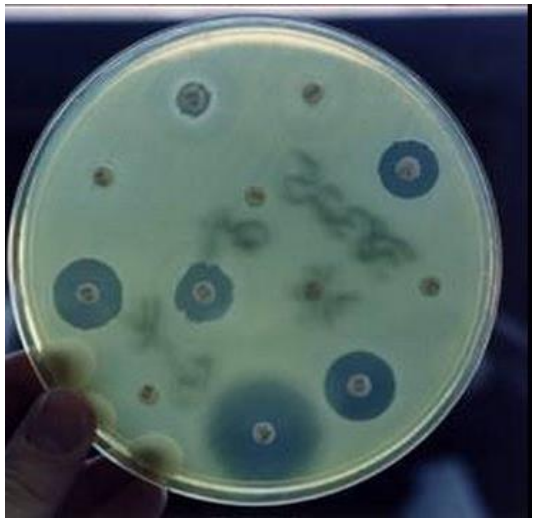
  - Micromonospora purpurea
  - Nocardia mediterranei
  - Streptomyces griseus
- Fungi

  - Cephalosporium acremonium
  - Penicillium chrysogenum
  - P. griseofulyum

# Processing an Antibiotic

Biotechnologists induce mutations by inserting extra-species gene sequences while

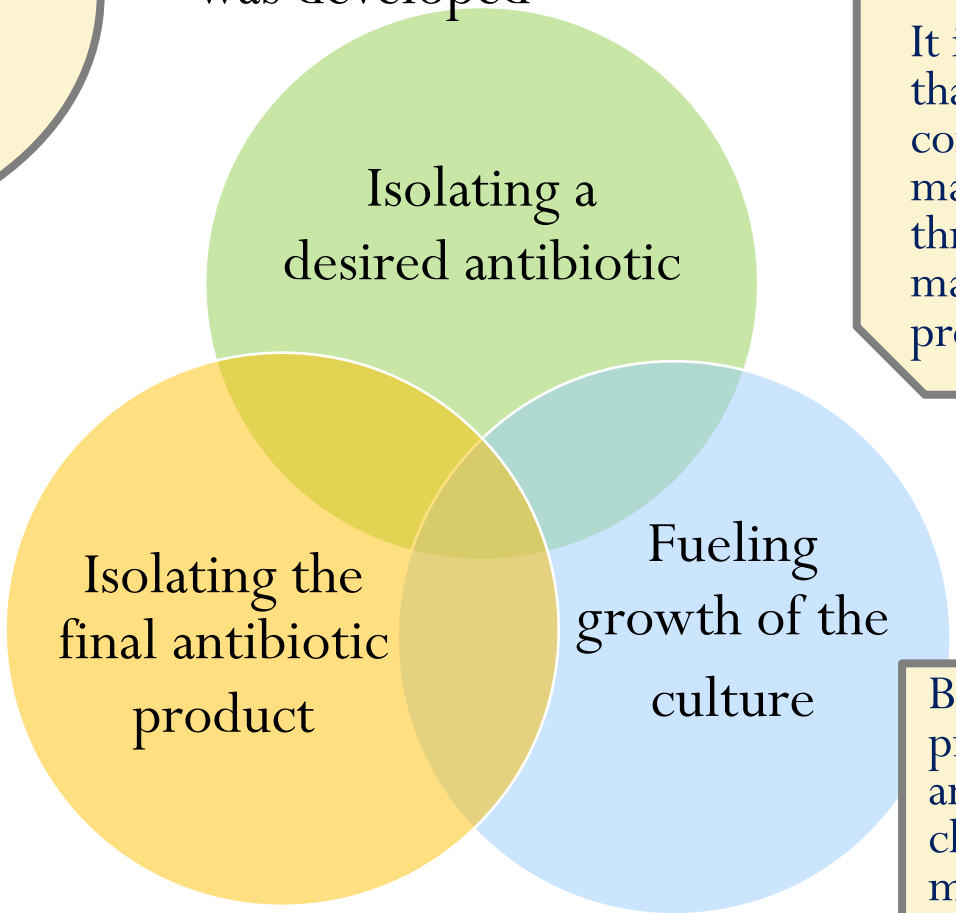
Screening procedures enable the scientist to isolate the correct mutant



# Processing an Antibiotic

Although most antibiotics occur in nature, they are not normally available in the quantities necessary for large-scale production.

For this reason, a fermentation process was developed




It is important that sterile conditions be maintained throughout the manufacturing process.

Contamination by foreign microbes will ruin the fermentation.

Biotechnologically produced antibiotics are chemically modified strains of *Penicillin*

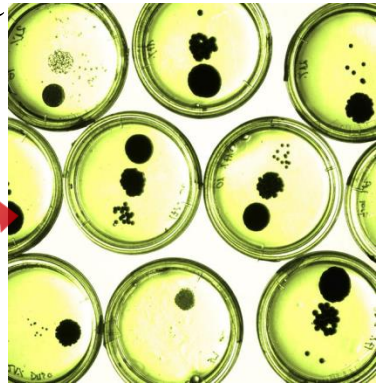
# How to Isolate a Desired Antibiotic

Results in a zone of inhibition once it is exposed to it


Microorganisms are kept in Erlenmeyer flask

Aseptic transfer of about 1 $cm^3$

Selecting an individual colony



Transferring it to several sterile petri dishes

Drying and incubating for about 48 hours

A single colony of the unknown organism is transferred.

Further incubating

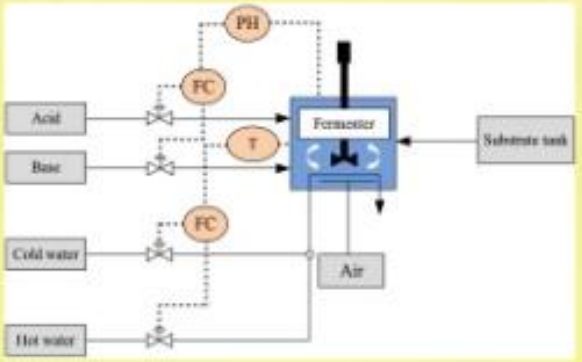
Screening by extracting a sample with a selected contaminant that is sensitive to a particular antibiotic

# Upstream processing

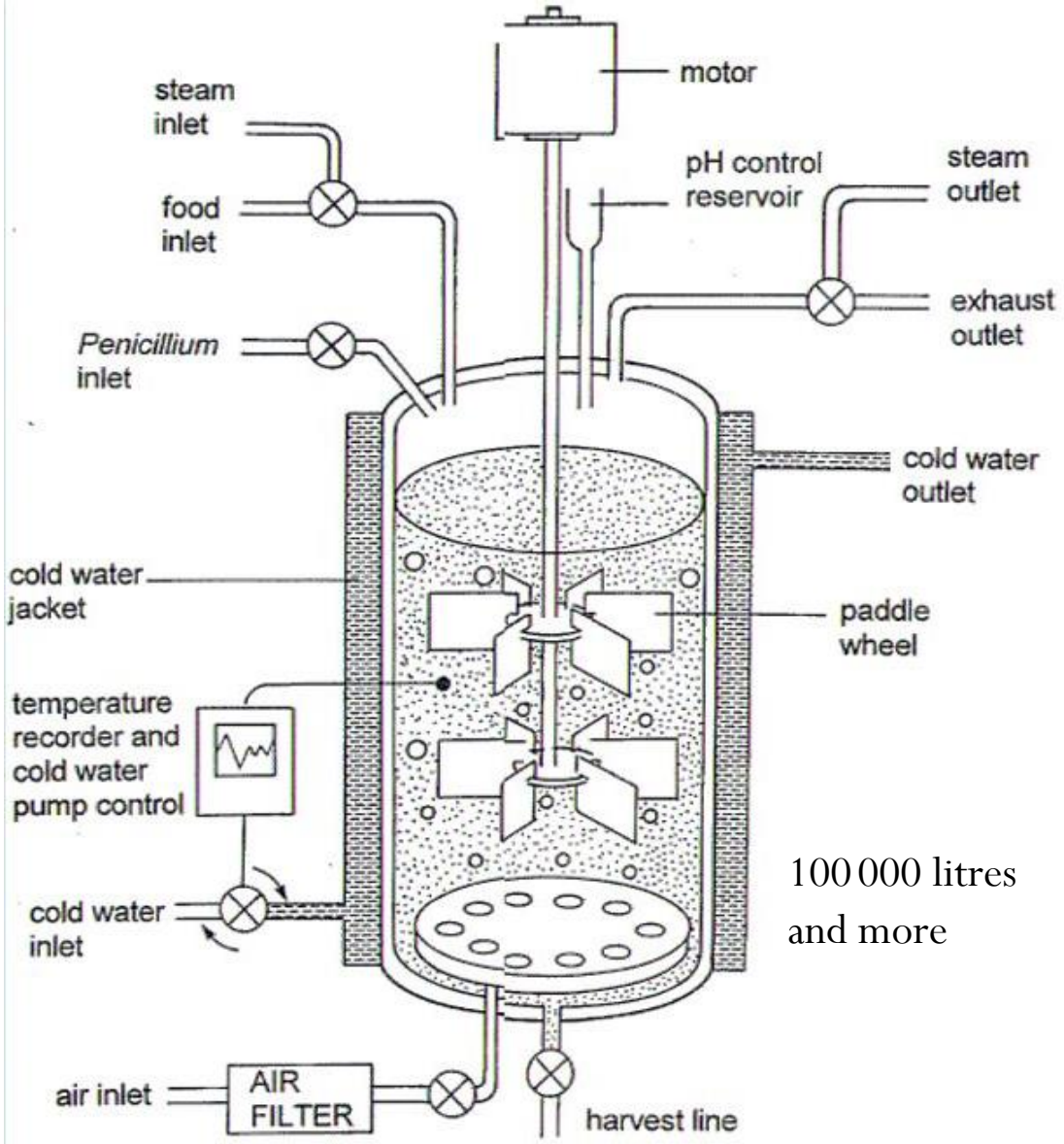
- Uses any technology that leads to synthesis of product
- Production strains stored in dormant form
- Incubated and fermented with oxygen to grow
- Uses carbon, nitrogen, and mineral sources in penicillin production

# Fermentation

- Inoculum seeded in fermenter
- Temperature, pH, and mineral contents controlled
- Incubated with oxygen to grow
- Antibiotic produced over time



# FERMENTATION in Antibiotics' Produsing.




100 000 litres and more

The fermentation conditions with such as temperature, PH, mineral contents should also be maintained.

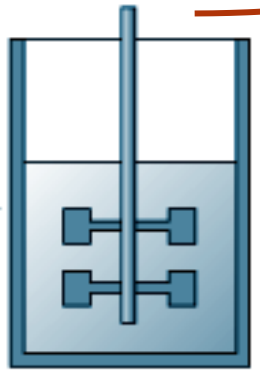
The process starts immediately after the addition of the inoculum strain according to batch procedure.

After the required amount of time, the required antibiotic is produced in the fermenter.

# Downstream processing

- Extraction and purification of biotechnological product from fermentation
- Stages:
  - Solid-liquid separation
  - Extraction
    - Filtration, rotary vacuum filter
    - Add to low pH solution
  - Purification
    - Charcoal treatment
    - Sterilization
    - Crystallization
- Packaging:
  - Sterile vials as powder or suspension
  - Tabletted with film coating

# Processing an Antibiotic



Fermentor

Solid

Preliminary Treatments

Filtrating

Adsorbtion
Cemical Precipitation
Solvent extraction

Extract

Extraction

Partially purified antibiotic in solutio

Waste solid is filtered, sterilized

Recrystallization
Purification

Dryer

Antibiotic

Animal feed supplement

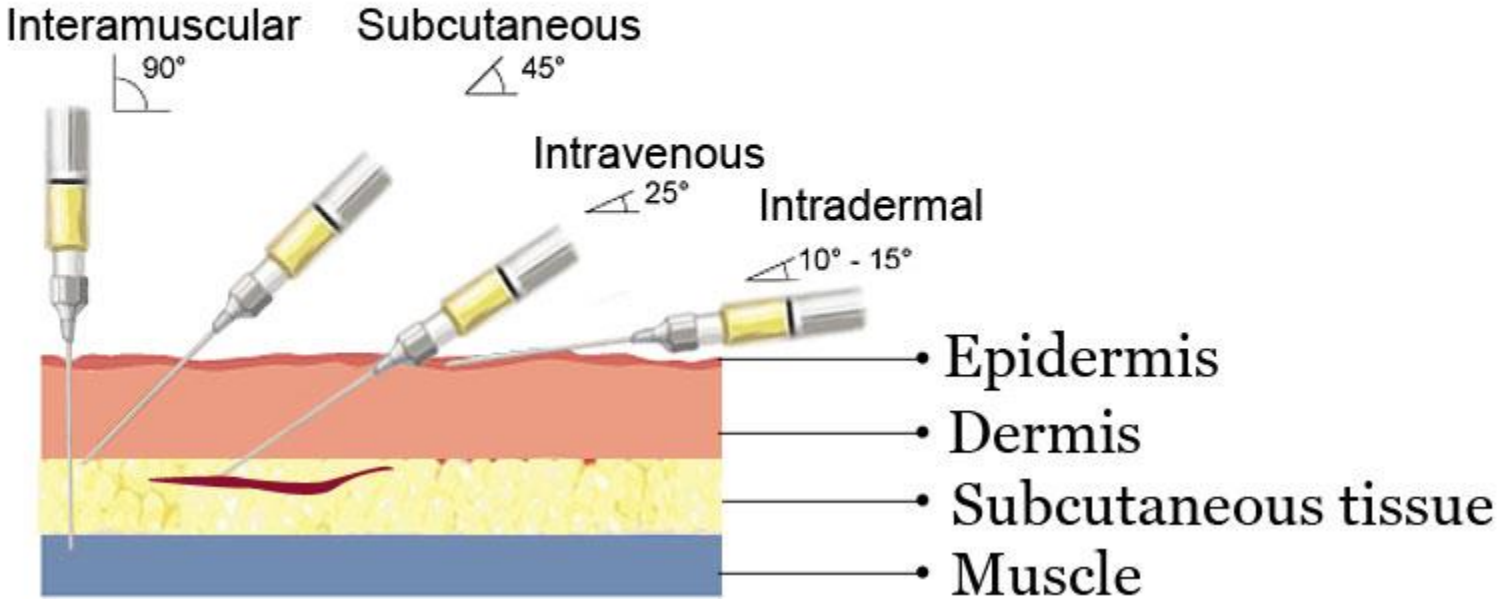
In order to be produced in a manufacturing scale a new antibiotic must to be low cost, unicable, defferent from similar samples, high effective.

# How medicines are made?

# Use of Antibiotics: administration

- The type of administration affects the absorption rate of the drug
- Parentally (intravenously, intra-muscularly…)
  - Quickest absorption, more serious cases
  - IV less painful, less irritating, higher dose
- Orally (tablets or caplets)
- Local application to skin, mucous membranes, respiratory tract…
- Inhalation as aerosol (small molecules sprayed in respiratory passages)

# Types of Parenteral Routes of Administration



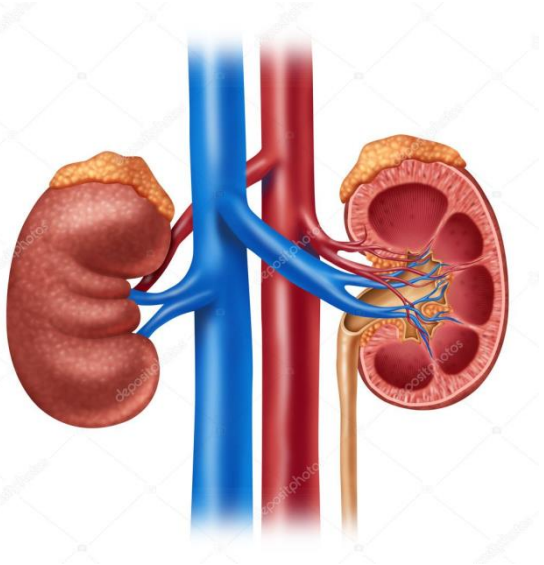

Orally

Inhalation as aerosol

Local application to skin

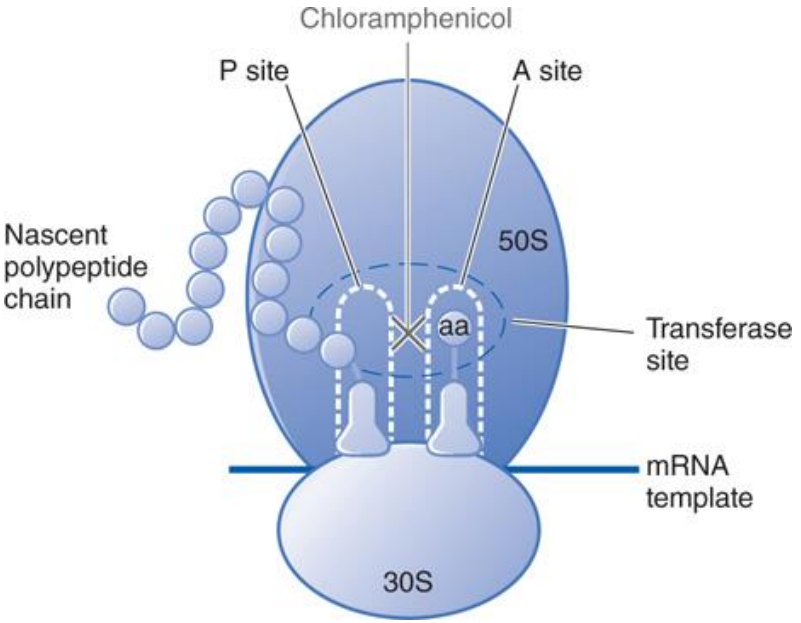
# Phisiological effects

**Metabolized by:**
Kidney (most important);
Also excreted through faeces, sweat, respiration.

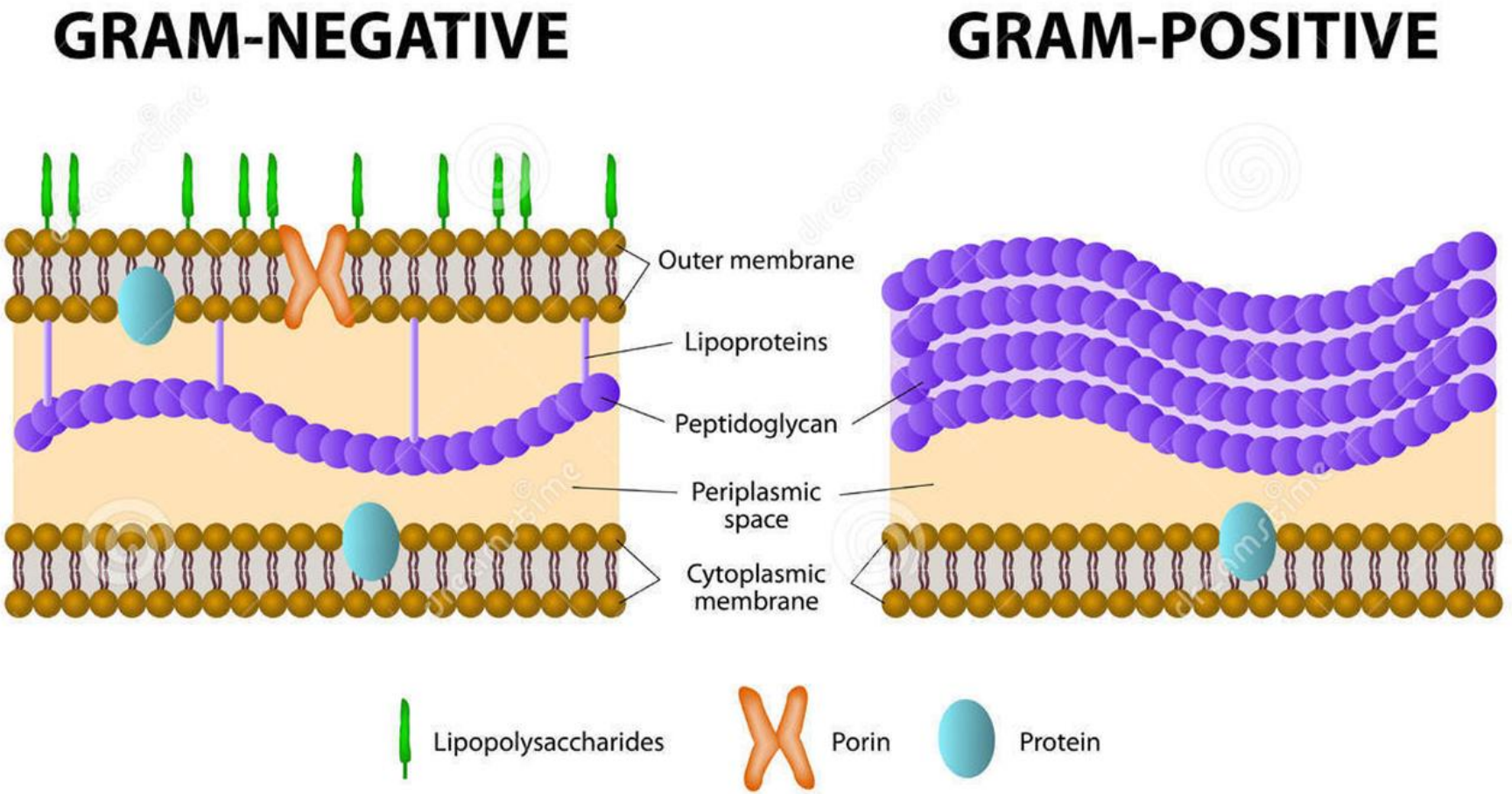


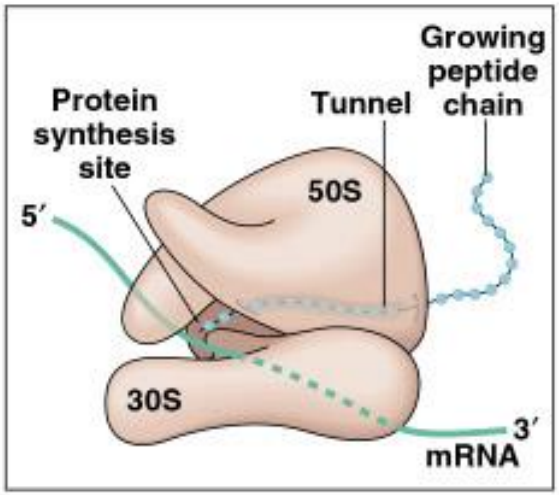
**Cell wall synthesis inhibitors:**
Prevent microorganisms/bacteria from making peptidoglycan for cell wall.

**Interfering with protein synthesis:**
Inhibit protein synthesis machinery (tetracyclines, chlormphenicol, aminoglycosides, macrolides).

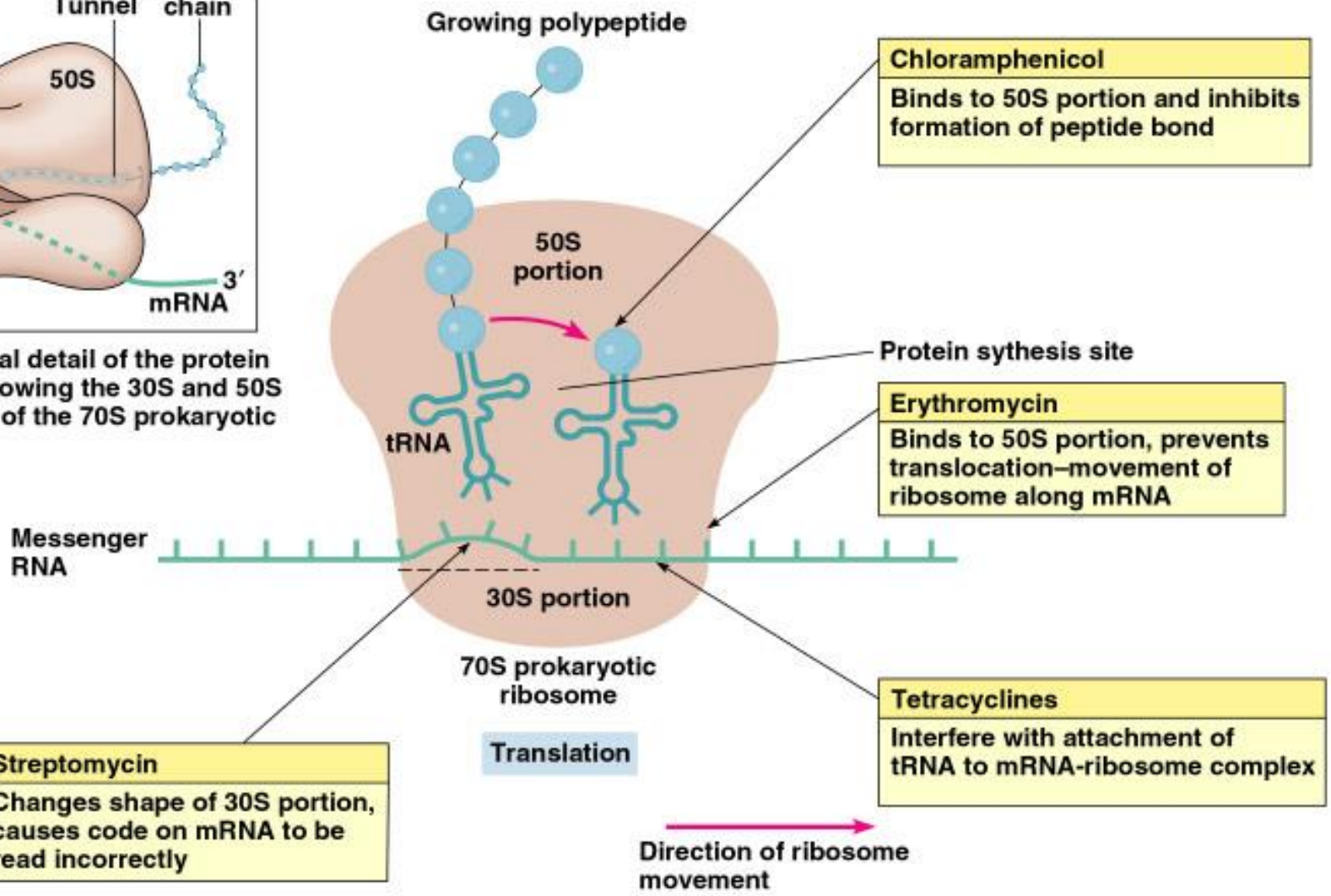


# Peptidoglycans for bacteria's cell wall





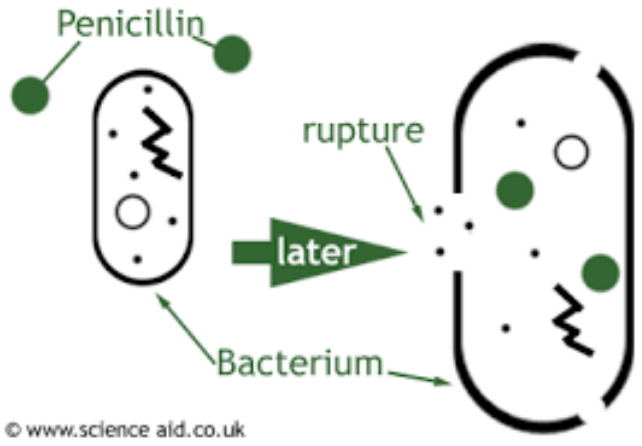

**(a)** Three-dimensional detail of the protein synthesis site showing the 30S and 50S subunit portions of the 70S prokaryotic ribosome.



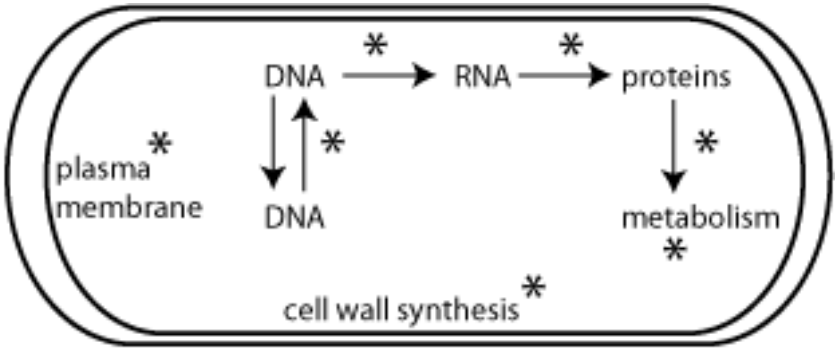

**(b)** In the diagram the black arrows indicate the different points at which chloramphenicol, erythromycin, the tetracyclines, and streptomycin exert their activities.

# Phisiological effects

**Cell membrane inhibitors:**
Disrupt integrity/structure of cell membrane, killing them (Polymyxins); Effective on Gram-negative bacteria that have definite cell membrane.




**Effect on Nucleic Acids:**
Some bind to the proteins required for processing of DNA and RNA, stopping synthesis and growing of cells (Quinolones, rifamycins)



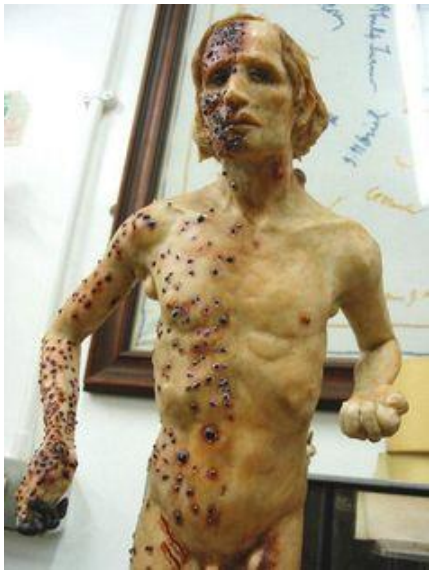

Antibiotics act at all of these points

# Phisiological effects

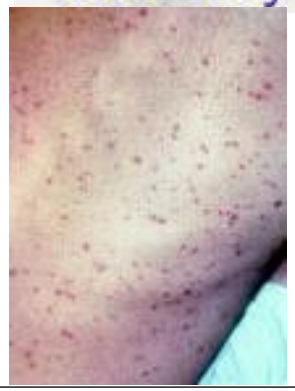
**Competitive inhibitors:**
Anti-metabolites/growth factor analogs. Competitively inhibit the important matabolic pathways in bacterial cell (Gantrisin, Trimethoprim)


# Application of Antibiotics

- Streptomycin effective against diseases that penicillin was not (eg. Bubonic plague)
- Aureomycin does work of both penicillin and streptomycin
- Terramycin considered one of most effective antibiotics ever found
- Chloromycetin effective against typhus, whooping cough, and typhoid
- Fluoroquinolone used on chickens and turkeys to stop E. coli deaths
- Clinically used in ophthalmology

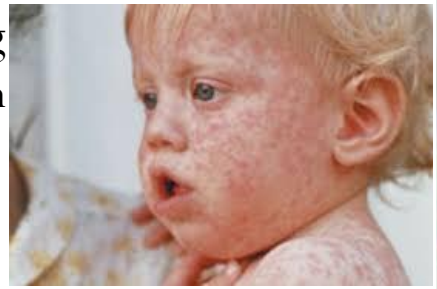


Bubonic plague



Typhys

Whooping cough



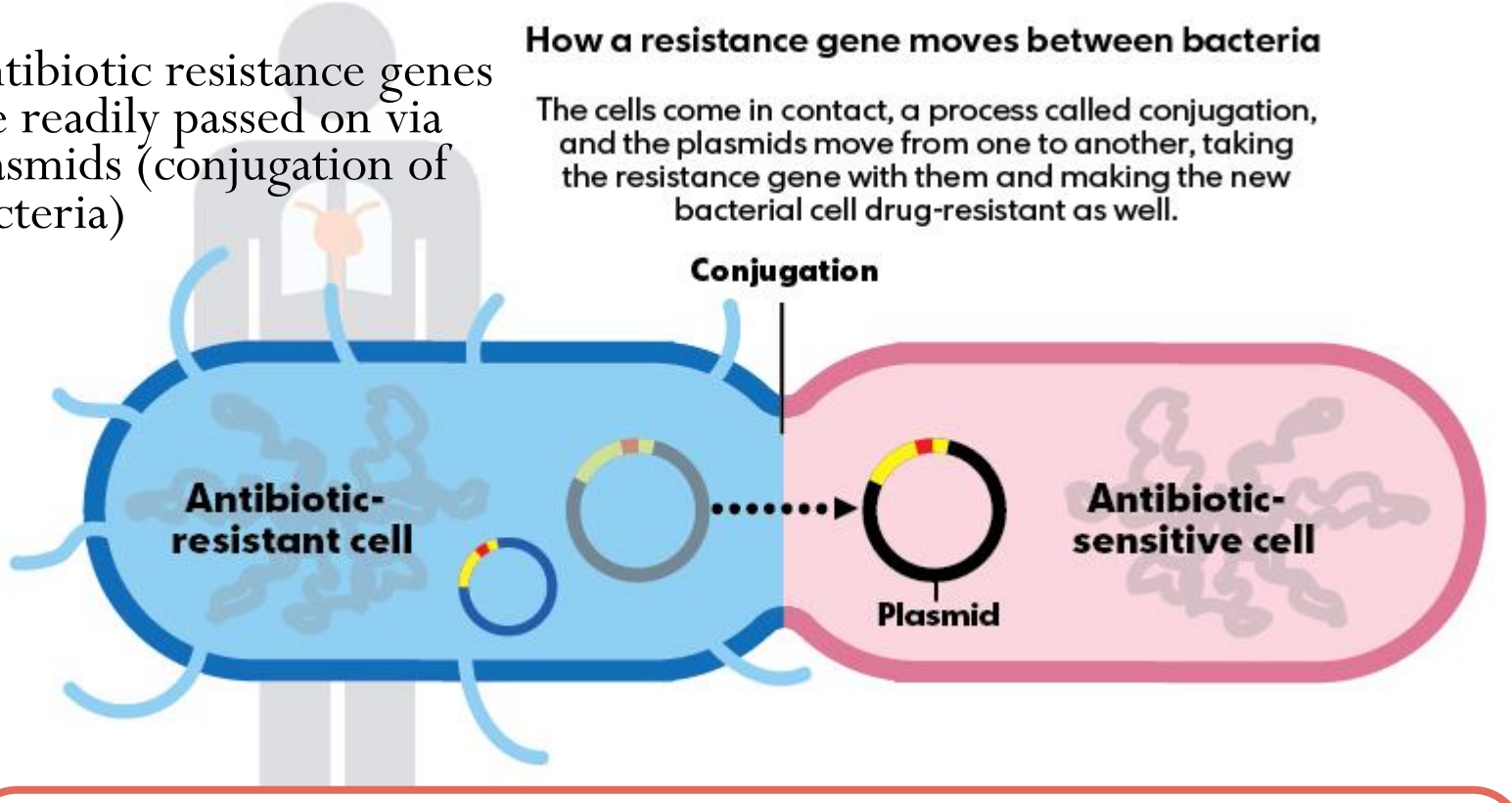
# Antibiotic resistance

## Genetically

- Evolutionary process based on natural selection
- Newer strains of bacteria resistant to previously lethal antibiotics
  - Antibiotics act as selective pressure on bacteria
  - Those that survive are selected for, and go on to reproduce
- Instrinsic resistance can occur naturally from genetic makeup
- Mutations also aid survivorship
- Cross-resistance with other bacteria lead to co-resistance of many antibiotics

# Antibiotics cross-resistance

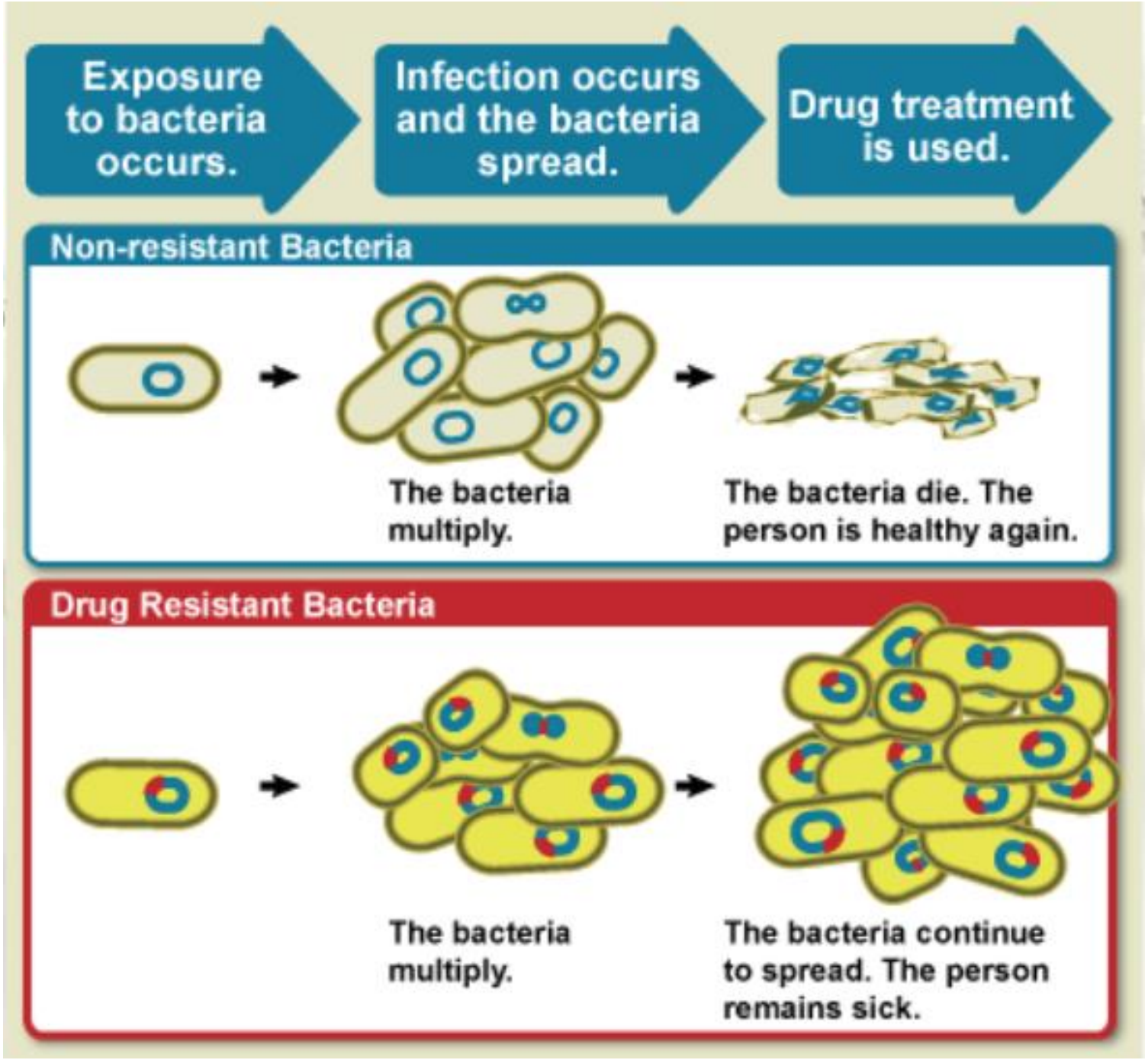
Antibiotic resistance genes are readily passed on via plasmids (conjugation of bacteria)




The presence in the plasmid of antibiotic resistance genes plays a significant role in isolating bacteria with an embedded portion of foreign DNA.

# Mechanisms of Antibiotics resistance

- Microorganisms may lack the structure on which the antibiotic acts (for example, bacteria of the genus mycoplasma (Latin Mycoplasma) are insensitive to penicillin, since they do not have a cell wall);
- Some microorganisms are impervious to certain antibiotics (most gram-negative bacteria are resistant to penicillin G, in which the cell wall is protected by an additional membrane);
- Microorganisms can translate the antibiotic into an inactive form (many staphylococci (Latin Staphylococcus) contain the β-lactamase enzyme which destroys the β-lactam ring of most penicillins);
- As a result of gene mutations, the metabolism of the microorganism can be modified in such a way that the chemical reactions blocked by the antibiotic are no longer critical to the body's vital functions;


Exposure to bacteria occurs.
Infection occurs and the bacteria spread.
Drug treatment is used.
Non-resistant Bacteria
The bacteria multiply.
The bacteria die. The person is healthy again.
Drug Resistant Bacteria
The bacteria multiply.
The bacteria continue to spread. The person remains sick.

# Superbacteria

# Strains

Antibiotic use in foods has led to creation of antibiotic-resistant strains

- Salmonella spp.
- Campulobacter spp.
- Escherichia coli
- Enterococcus spp.

# Solutions

- Development of pharmaceutical compounds that reverse antibiotic resistances
  - Called "resistance modifying agents"
- Idea of using bacteriophages instead of antibiotics
  - Phages tailored to specific bacteria, to destroy them