Introduction to Infection

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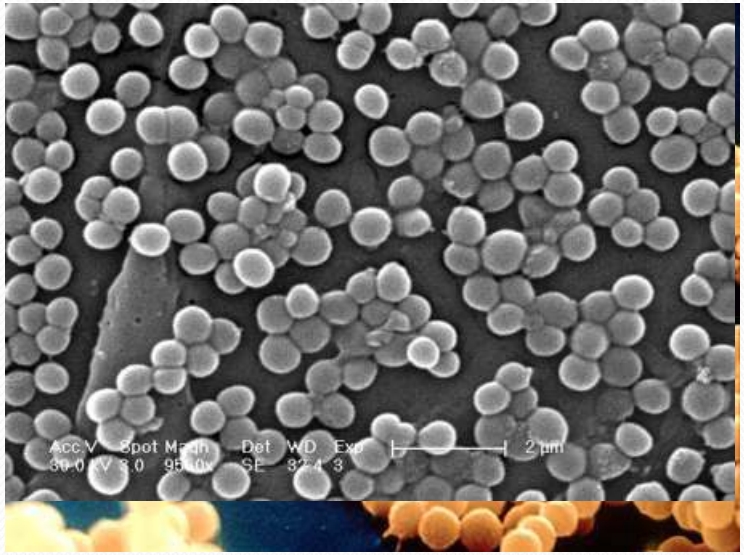
Introduction

- Bacteria
- Gram Stain
- Antibiotics and mechanism of action.
- MRSA
- Biofilm

Bacteria

- *Bakterion*: little staff/rod.
- Prokaryotes
- Sized in micrometers (μ m)

- Morphology
  - Bacilli, Cocci, Vibrios.
  - *streptos, staphule*.

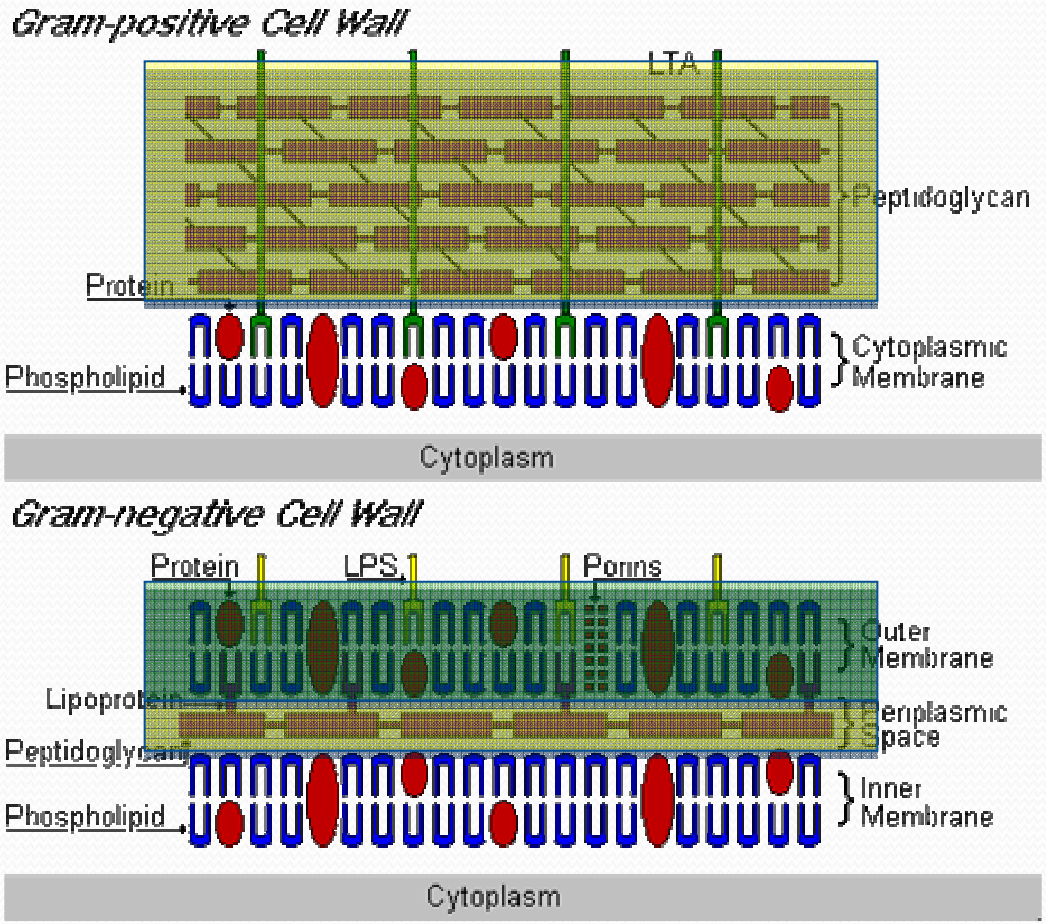


Gram Stain

- Christian Gram (1853-1938)
- *Streptococcus pneumoniae* and *klebsiella pneumoniae*

	Gram +	Gram –
Before staining		
1° stain (crystal violet)		
2° stain (Lugol's iodine)		
Solvent wash (acetone)		
Counterstain (safranin)		

Cell Wall Differences



Common Bacteria

Gram +ve Cocci	Gram –ve Cocci	Gram +ve Bacillus	Gram –ve Bacillus
Staphylococcus aureus *S. epidemidis* *aeruginosa*	*Neisseria gonorrhoea*	*Clostridium tetani* *Clostridium perfringens*	*Pseudomonas*
Enterococcus spp.			*Eikenella corrodens*
Streptococcus spp. *influenza*			*Haemophilus*
			Escherichia coli
			Salmonella typhi

Osteomyelitis

- Newborn - *S. aureus,* Gp B Strep.
- Children < 4 years – *S. aureus,* Gp A Strep.
  - *H. influenza* now very rare!
- Adults – *S. aureus,* various.
- Sickle Cell – *Salmonella.*

Septic Arthritis

- Newborn – *S. aureus*, Gp B Strep.
- Children 3-14 years – *S. aureus, S. pyogenes, S. pneumonia,* (*H. influenza*)
- Adults – *S. aureus, N. gonorrhoea, Strep.*

Bite Infections

- Human – *Steptococcus viridans, Eikenella*
- Dog/Cat – *Pasteurella multocida*

Miscellaneous

- Chronic, low-grade infections – TB.
- Marine injuries – *Mycobacterium marinarum*
- Necrotising fasciitis – Strep, Coliforms, and Clostridia.
- Toxic Shock Syndrome = Toxaemia.

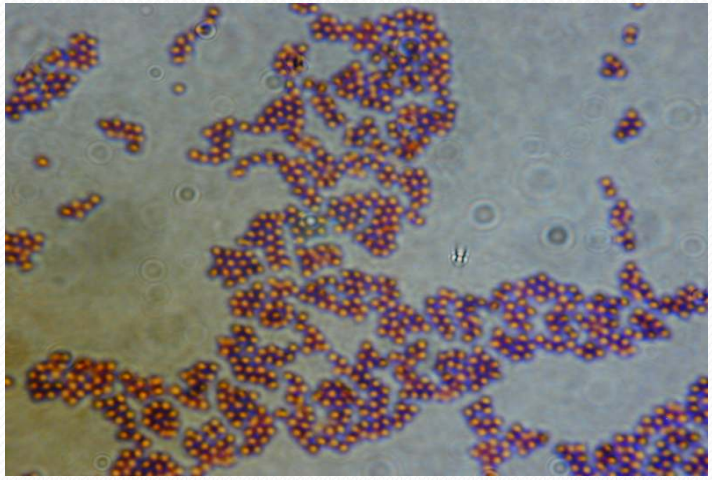
Prosthetic Infections

- Early
  - < 3 weeks.
  - Thorough lavage and exchange of modular parts.
- Haematogenous
  - < 3 weeks from acute haematogenous seeding
  - As for early.
- Chronic
  - > 3 weeks from index op or haematogenous event.
  - 2 stage revision!

Prosthetic Infections

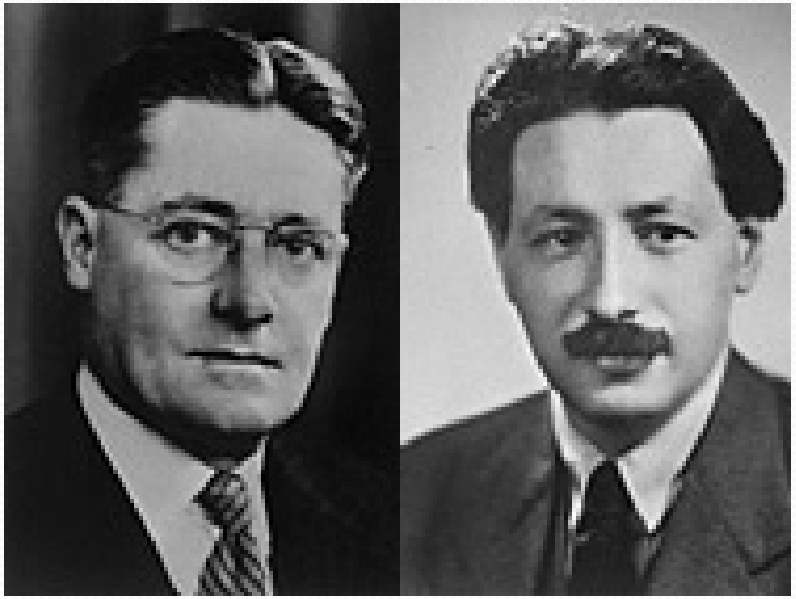
- 2 stage revision.
  - Interpositional PMMA spacer with high dose Abx
  - Reimplant 6/52 to 3/12.
  - BUT Interval determined by CRP, ESR and FBC.

- *S. epidermidis*
- *S. aureus*
- MRSA

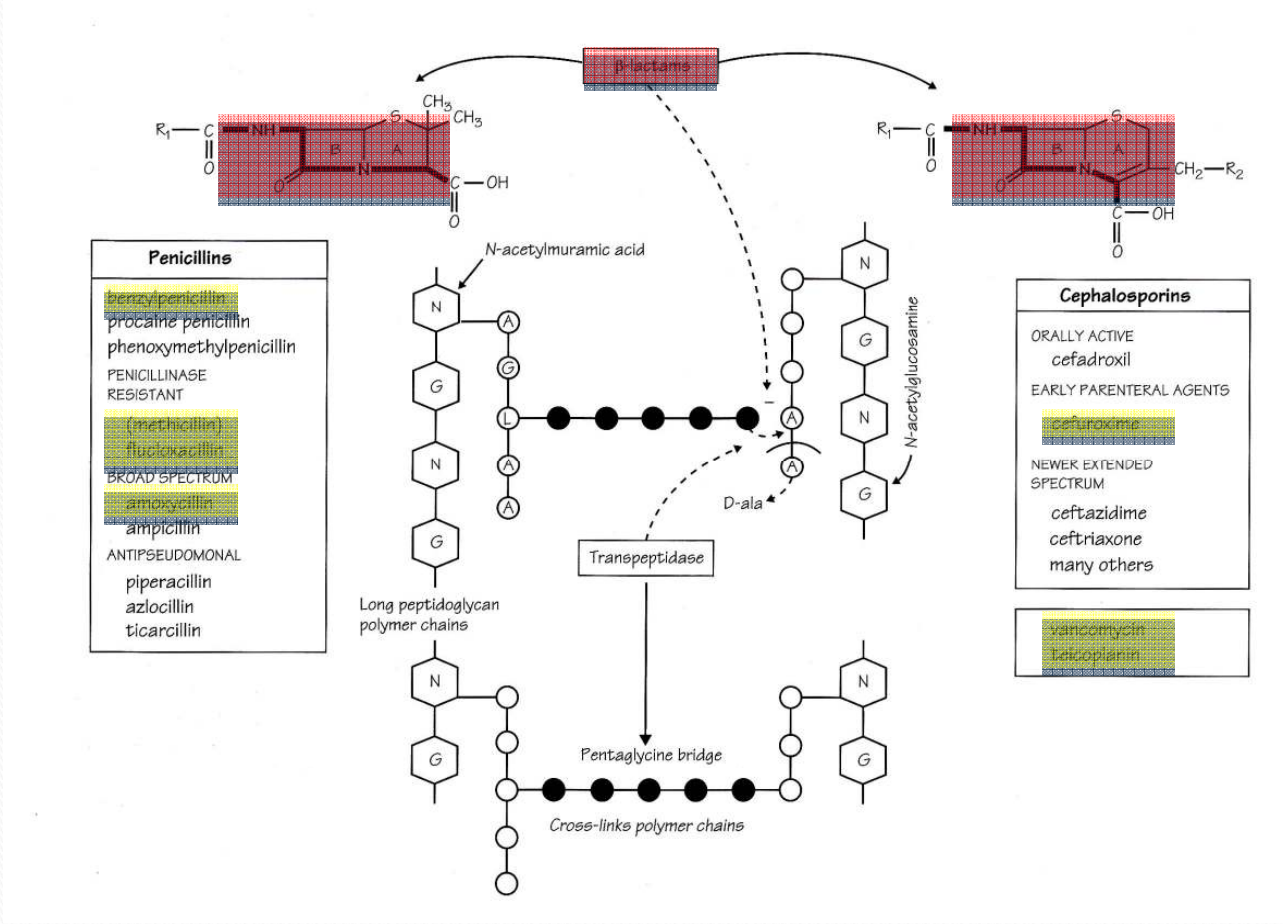


Antibiotics

- Sir Alexander Flemming (1928)
- Ernst Chain and Howard Florey (1940)
- *Penicillium* spores.



Antimicrobials that Inhibit Cell Wall Synthesis

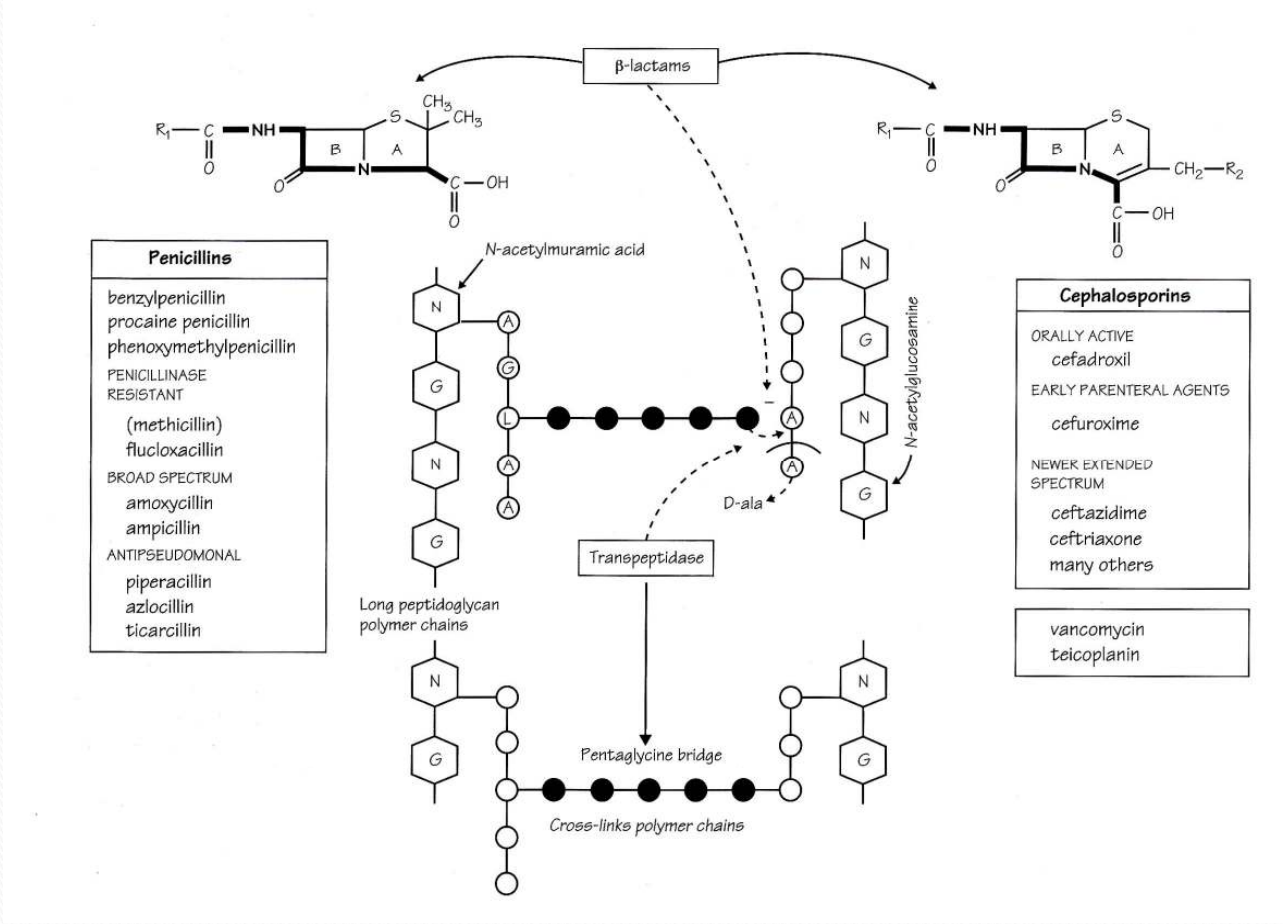


β -Lactams

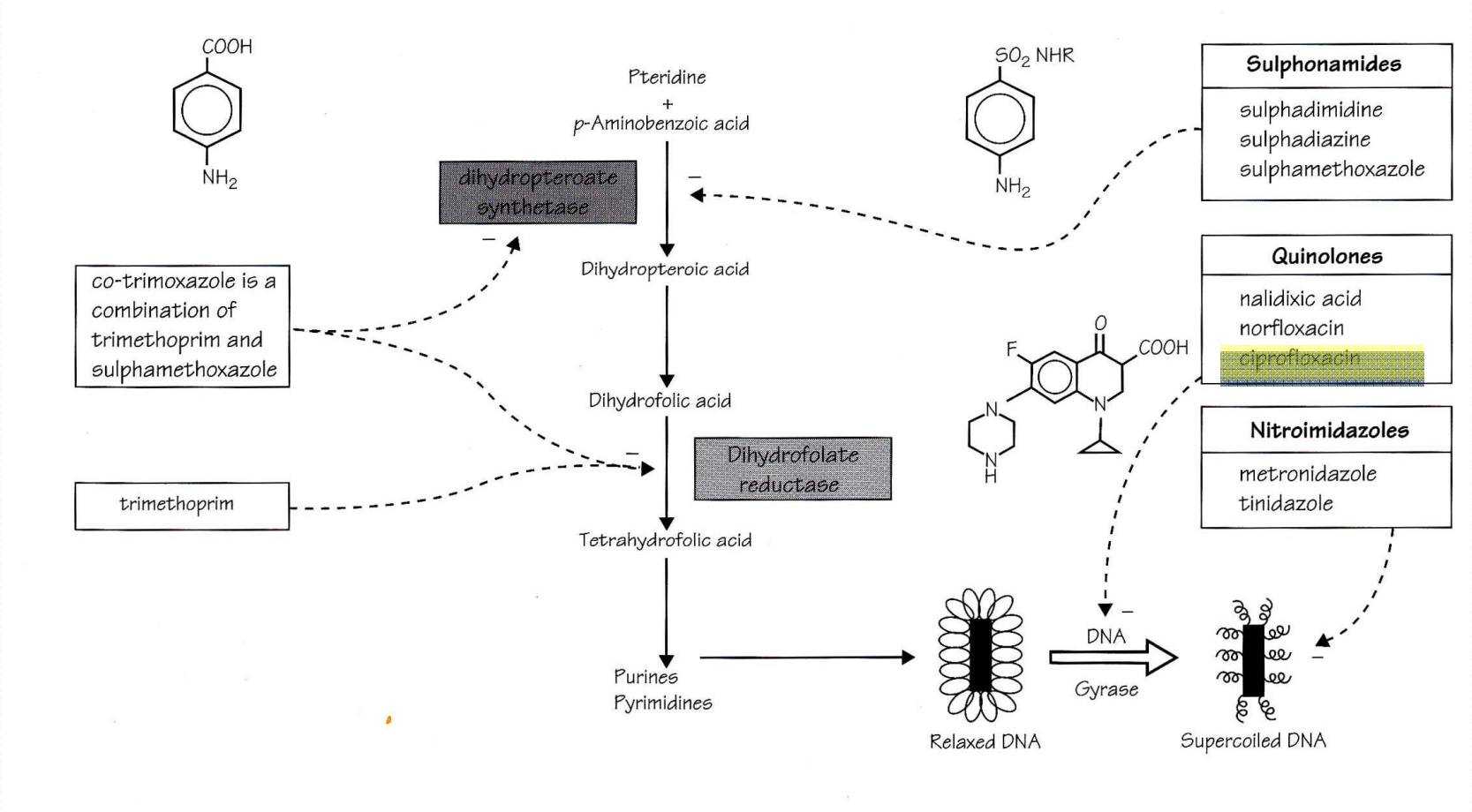
- Lactams are cyclical amides
  - Amide is a carbonyl (C=O) joined to a nitrogen.
  - β = 4 membered ring. 3 carbon, 1 nitrogen.

- Mimic *D*-alanyl-*D*-alanine.
  - Thus target transpeptidation of peptidoglycan synthesis.
  - Occupy the active site serine residue of PBPs

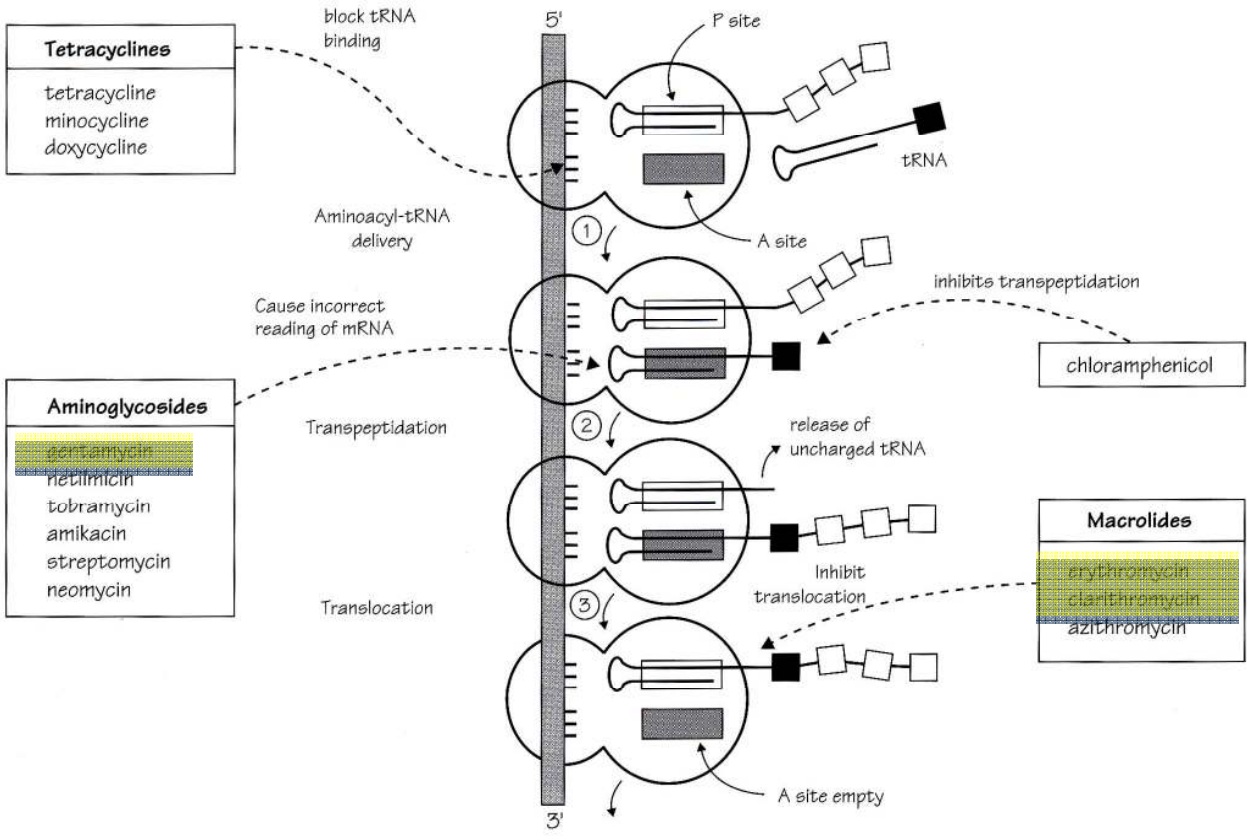
For Demonstration



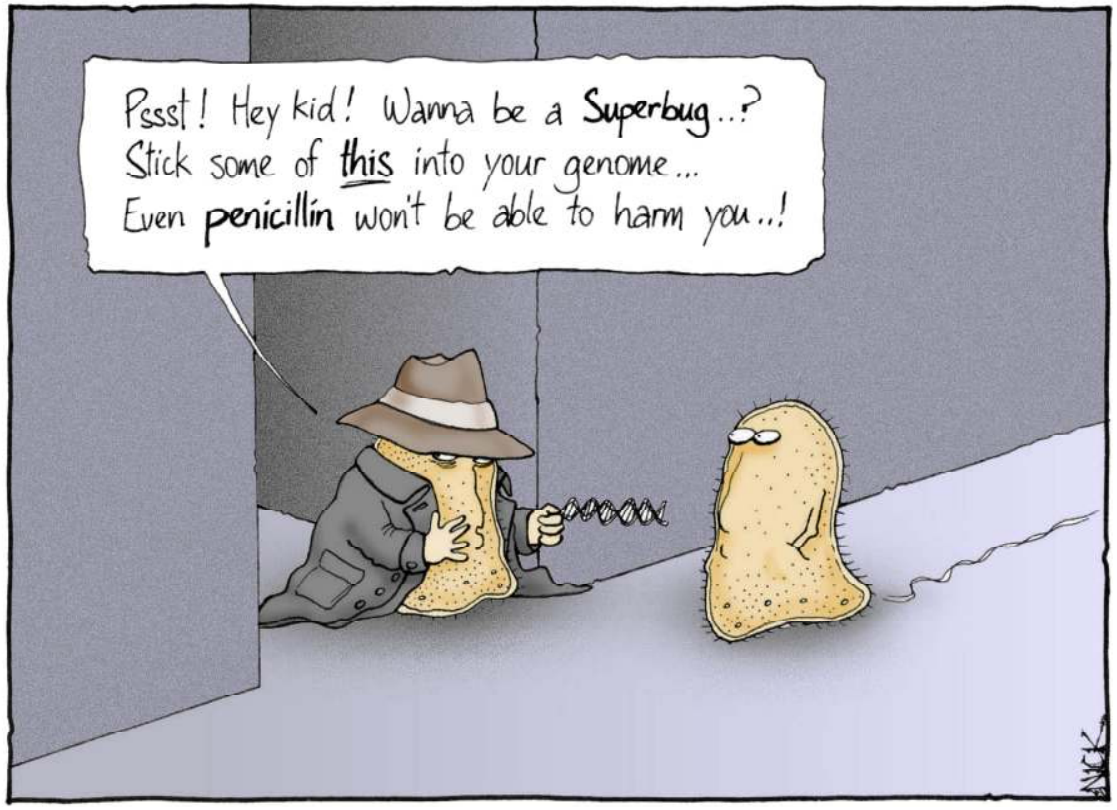
Antimicrobials that Inhibit Nucleic Acid Synthesis



Antimicrobials that Inhibit Protein Synthesis



Antibiotic Resistance

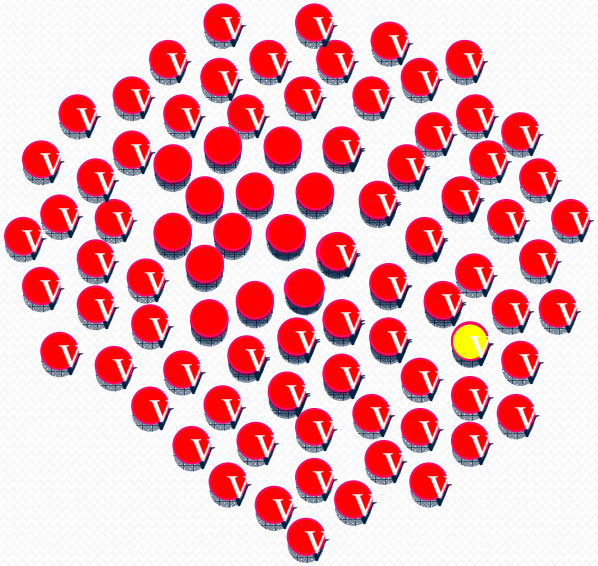


It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

Antibiotic Resistance

1. Inactivating enzymes that destroy the drug.
   - e.g. β -lactamase.
2. Decreased drug accumulation.
   - e.g. bact. cell membrane impermeable to tetracycline.
3. Alteration of binding sites.
   - e.g. Ribosomal mutations affect aminoglycosides.
4. Development of alternative metabolic paths.
   - e.g. Modified enzymatic pathways.

Selection



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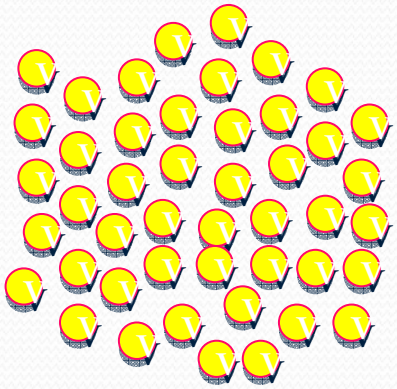
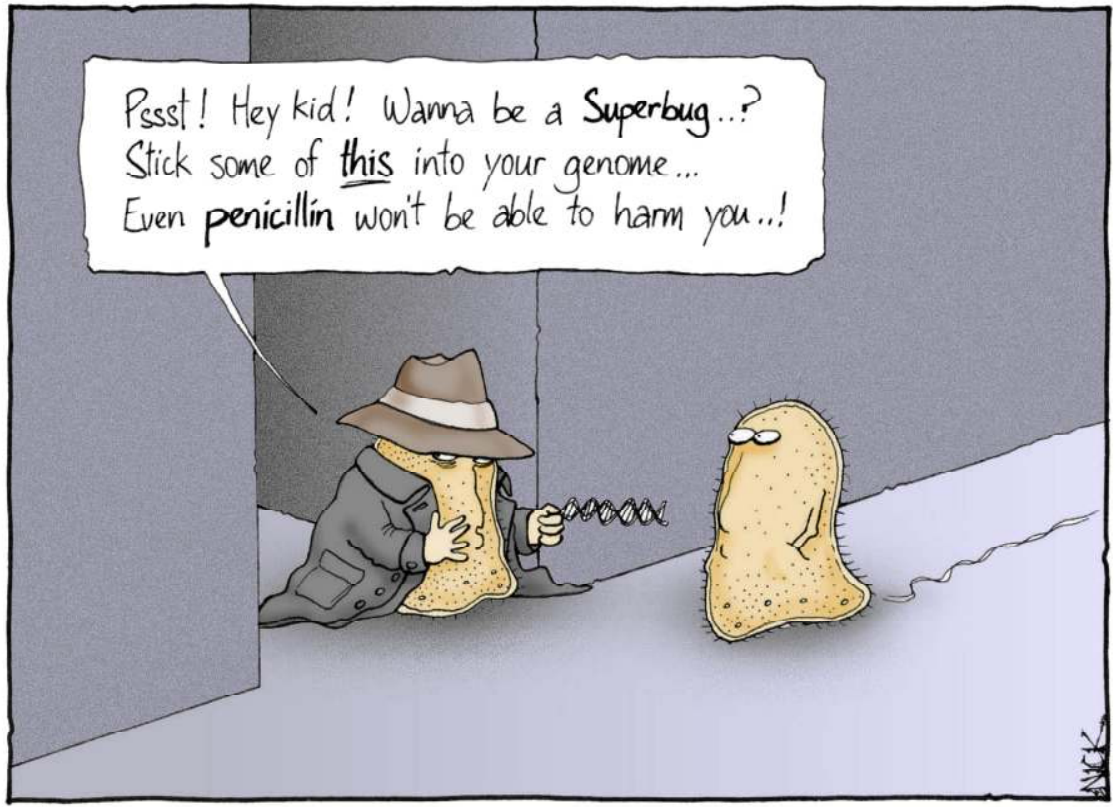


Transferred Resistance



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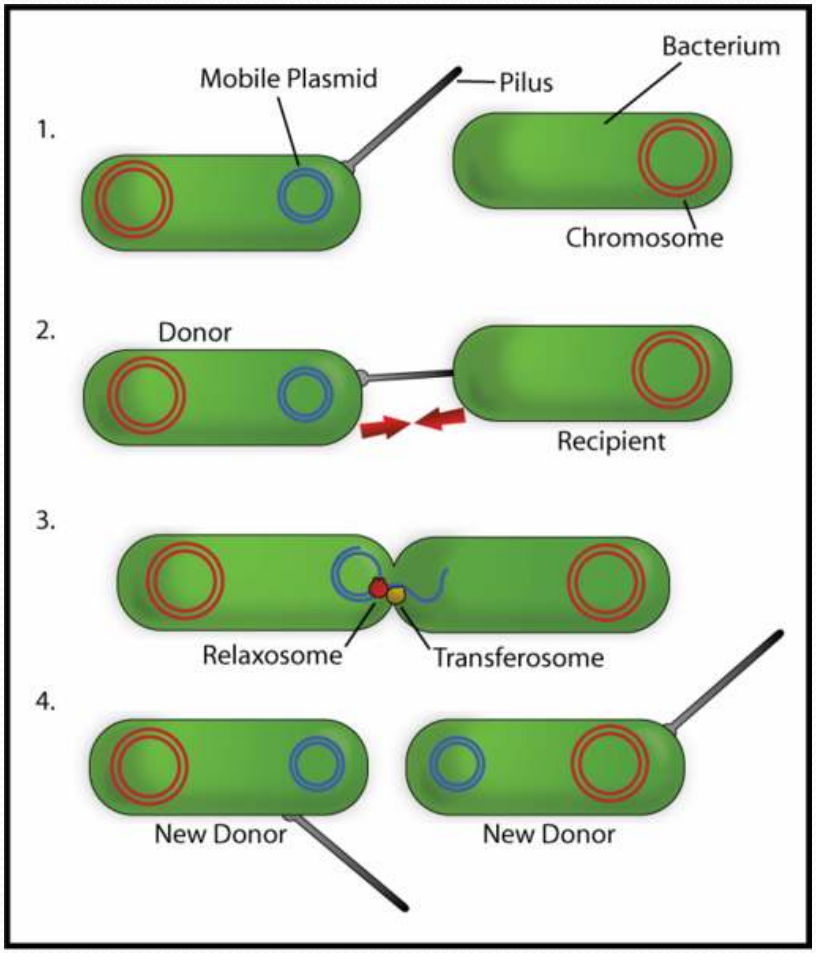
Transferred Resistance

- Plasmids.
- Transduction.
  - Relatively ineffective.
  - Staph, strep.
- Conjugation.
  - More effective.
  - Horizontal transfer!



Staphylococcus aureus

- Gram +ve, non-motile, non-sporing.
- Coagulase
  - Extracellular enzyme.
  - Breaks down fibrinogen to fibrin.

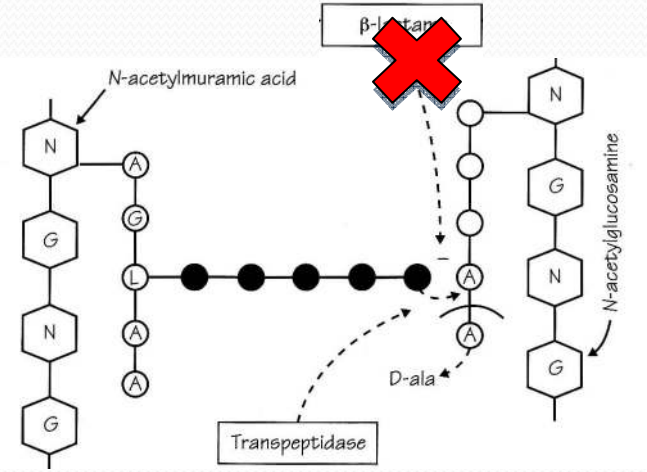
- Coagulase –ve *Staph*
  - *S. epidermidis*

MRSA

- *β* -lactamase.
  - Penicillin resistance.
- Alternative metabolic pathways.
  - Folic acid production – sulphonamide resistance.
- *mecA* gene.

mecA

- Altered penicillin binding protein.
  - PBP2a.
  - PBPs catalyse the glycan polymerisation reaction of peptidoglycan synthesis.



Biofilm

- Sessile, multicellular community.
- Bacteria embedded in a self-produced, hydrated matrix of:
  - Polysaccharide.
  - Teichoic acid.
  - Protein.
- Antibiotic and immune defence.
- Prosthetic Surfaces.

Biofilm

1. Surface attachment - “Early adhesion”.

2. Intercellular adhesion.

Surface Attachment

- **M**icrobial **S**urface **C**omponents that **R**ecognise **A**dhesive **M**atrix **M**olecules.
  - MSCRAMMs
  - FnbpA, FnbpB, ClfA, ClfB.
  - Bap.
  - Ebps.
  - Collagen adhesin. (*cna*)
  - SpA

Intercellular Adhesion

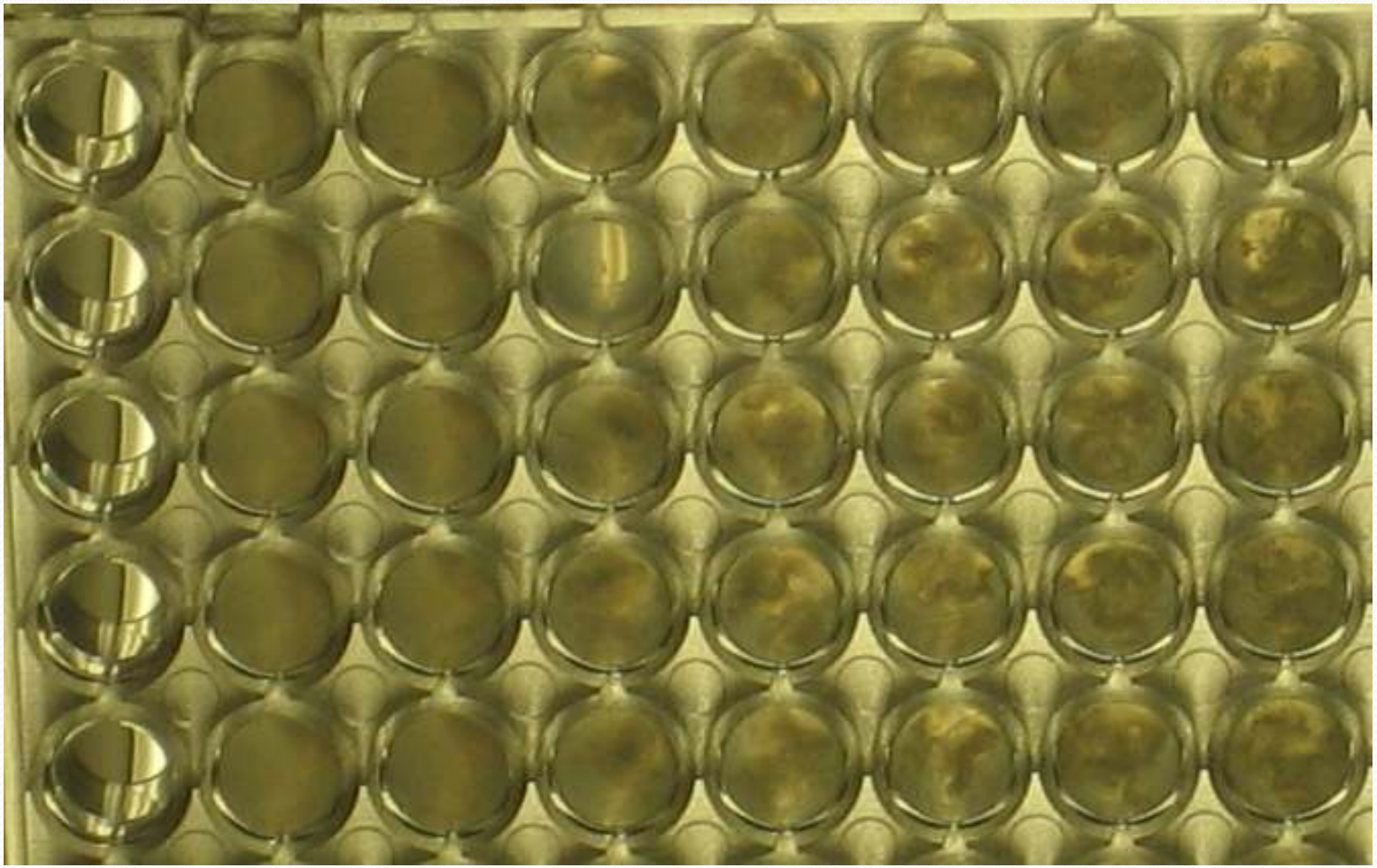
- PIA
  - Polysaccharide Intercellular Adhesin
    - *β* -1,6-linked *N*-acetylglucosaminoglycan (PNAG). *S. epidermidis.*
    - Poly-*N*-succinyl- *β* -1,6-glucosamine (PNSG). *S. aureus.*
    - PNSG – vaccination?
  - *ica* gene locus (*ica*ADBC and *ica*R)

Quorum Sensing

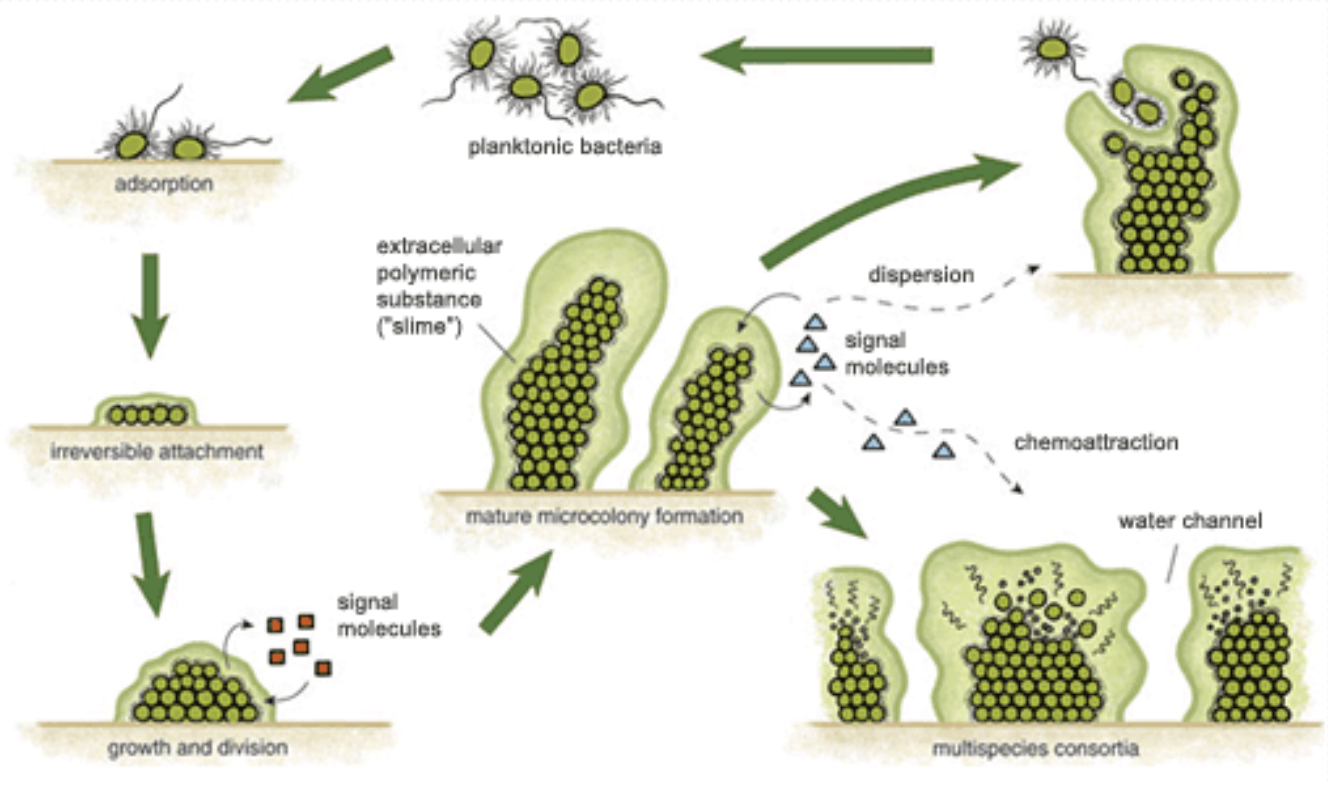
- Coordinated activity! Virulence.
- Auto-inducers
- Controlled by *agr* and *sar*.

- Initially surface proteins = adhesion and biofilm
- Then virulence factors = toxins, proteases, etc.

Biofilm



Biofilm


planktonic bacteria
adsorption
irreversible attachment
growth and division
signal molecules
extracellular polymeric substance ("slime")
mature microcolony formation
dispersion
signal molecules
chemoattraction
water channel
multispecies consortia

Summary

- Bacteria.
- Antibiotics.
- Antibiotic resistance.
- MRSA.
- Biofilm.

Sign on the Lab Door

Staph Only!

Mind Your Strep!

Stop Joking and *B. cereus*!

Any Questions?