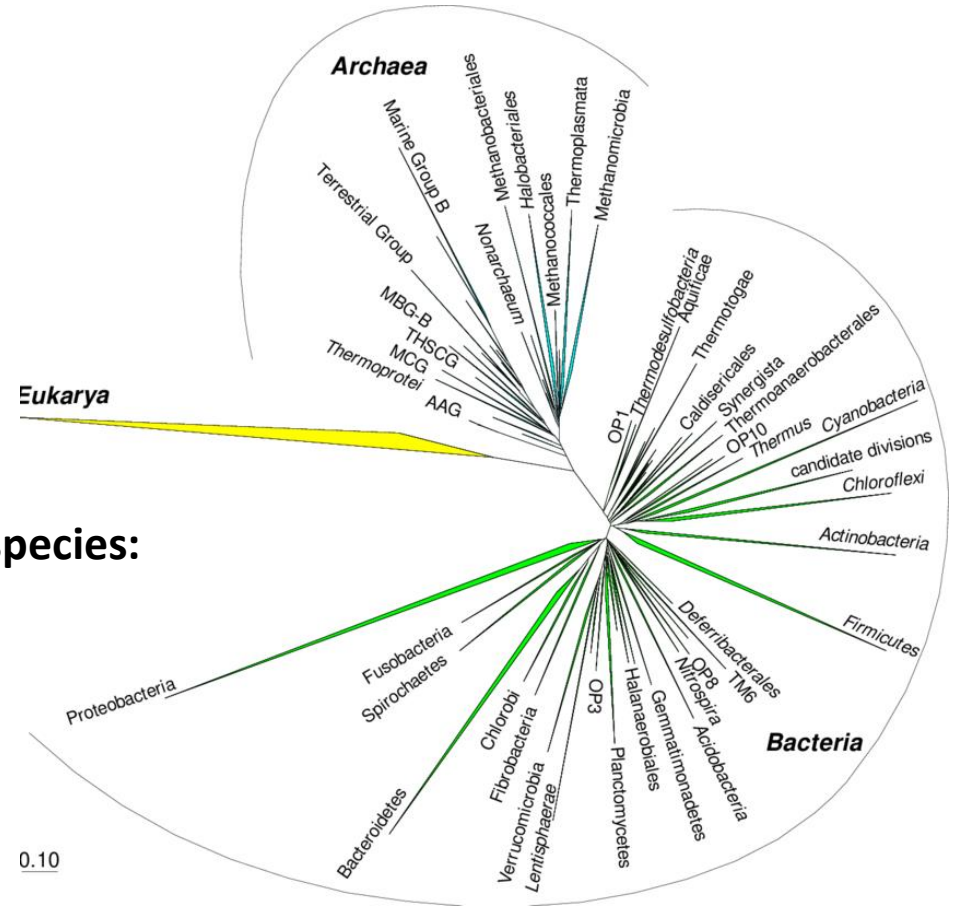
Bacterial cell and pathogenesis

Jan Tkadlec

Bacteria...

Bacteria
- **Unicellular**
- **Microscopic**
- **Prokaryotes (together with Archaea)**
- **Only a few are clinically significant**



Species described:

>30,000

(there are millions)

Clinically important species:

<50

Archea:

- extremophilic microbes
- probably an ancestor of eukaryotes (Asgard archea)
- not common pathogens
- BUT part of the microbiome (gut, mouth, skin, vagina...), they can be harmful!
- naturally resistant to ATBs

Medical bacteriology isn't a phone book in scope, it's more of a shopping list.

What does and doesn't make a bacterium

Bacteria do not have

membrane-separated organelles:
Nucleus and nucleolus = prokaryote
Mitochondria
Golgi apparatus
Endoplasmic reticulum
Lysosome
cytoscelet
does not form tissues (but biofilm)

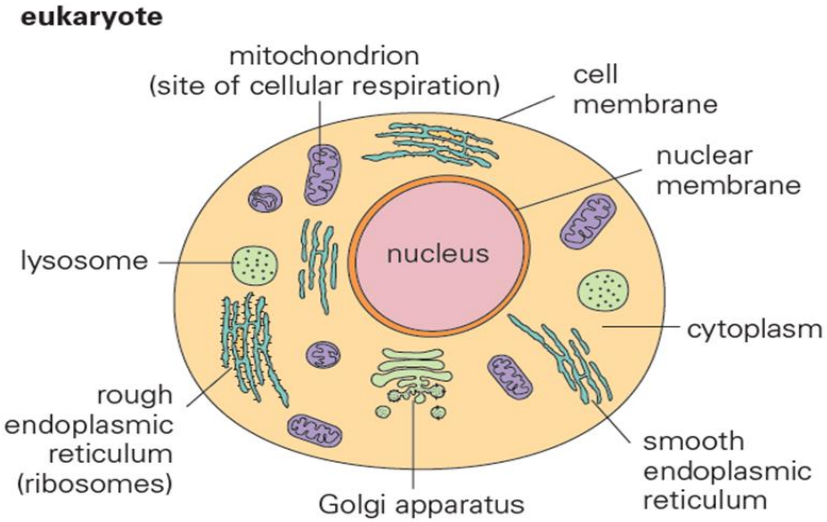
Bacteria have also (but different)

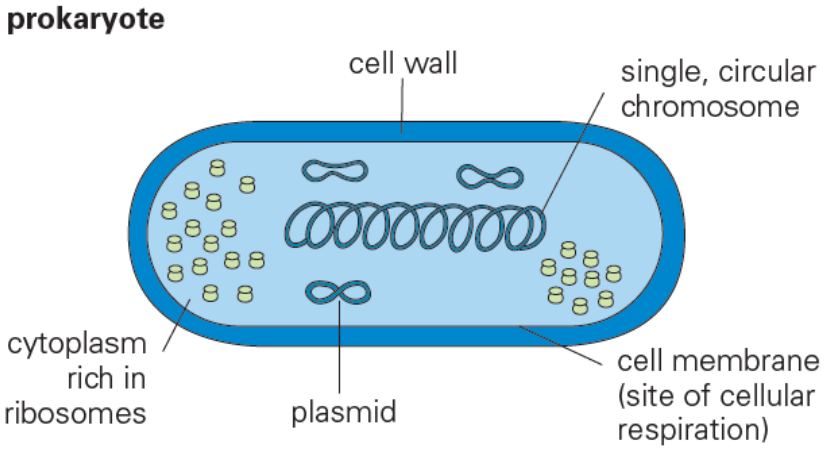
Ribozom
Flagella
Plasmids

Bacteria have: (mostly and not all)

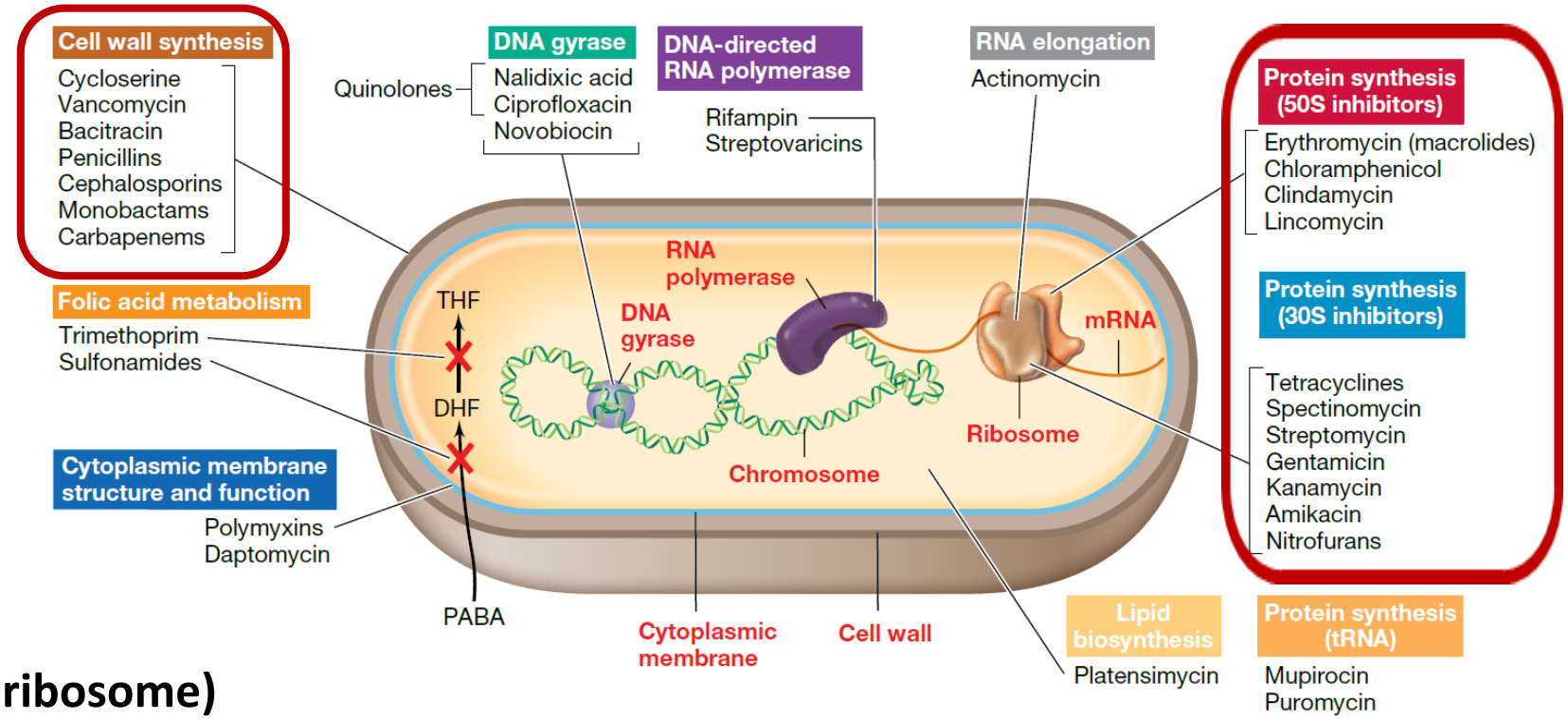
One circular chromosome
Cell wall made of peptidoglycan
Outer membrane (gram-negative)

Antibiotics and immunity





Targets of antibiotics in bacterial cell



<u>Targets of ATB:</u>

1. **Cell wall synthesis**
2. **Protein synthesis (ribosome)**
3. Synthesis and function of nucleic acids
4. Outer and cytoplasmic membrane
5. Synthesis of metabolites (folic acid)

Question: Why aren't there nearly as many antivirals as antibiotics?

Side note:
ATB targets processes in the growing cell!!!
i.e. it only kills the bacteria that is growing
therefore non-growing spores and slow growing bacteria (TB) are more resistant

Cell wall: Peptidoglycan

Exclusive to Bacteria: A unique biomarker and structural component.

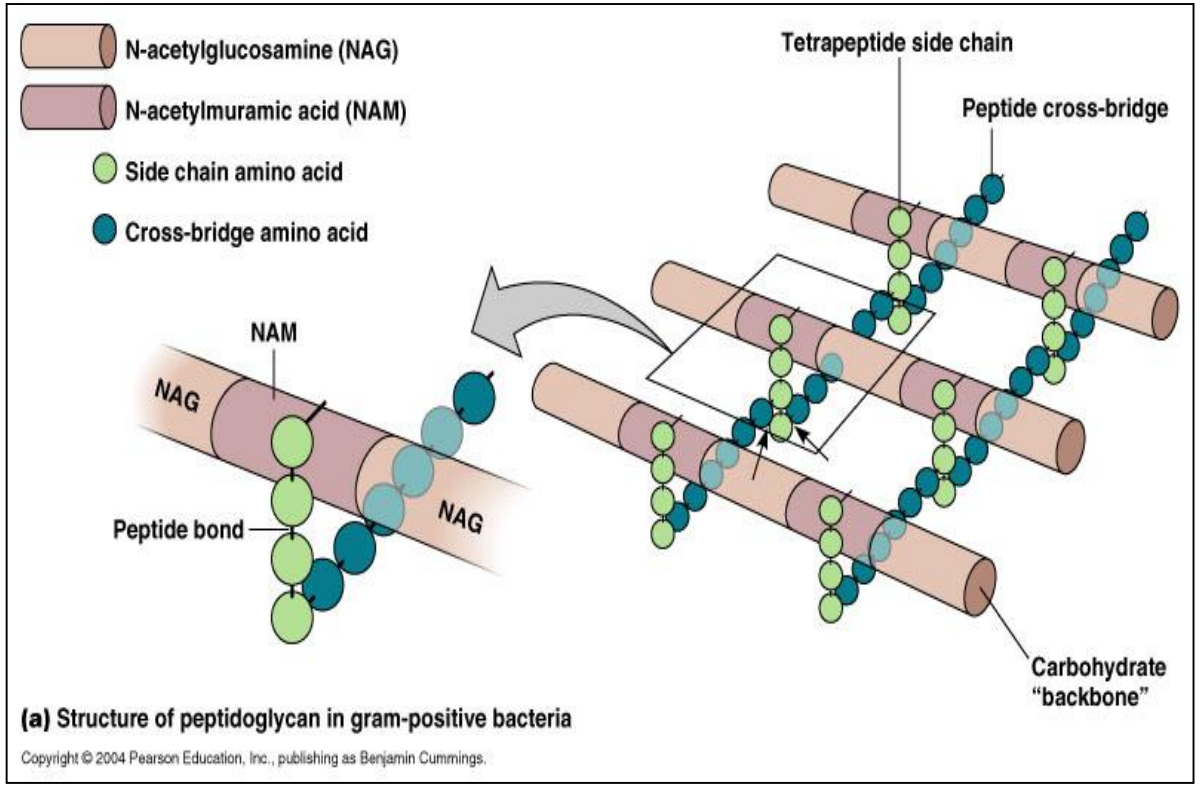
- **Physical Properties:** Provides a **rigid** framework while remaining **fully permeable** to nutrients and waste.
- **Core Function:** Maintains cell shape and prevents osmotic lysis (bursting) due to high internal pressure.

Protective Capabilities

- Peptidoglycan acts as a chemical and mechanical shield against:
  - **Physical Stress:** Mechanical damage and high intracellular pressure.
  - **Environmental Extremes:** Desiccation (drying out) and irradiation.

Molecular Architecture

- The structure is a complex, "net-like" macromolecule composed of:
  - **Polysaccharide Backbone:** Alternating units of **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)**.
  - **Peptide Cross-links:** Polymer fibers are stitched together via amino acid side chains, creating a high-tensile mesh.



Mycoplasma - bacteria without a cell wall

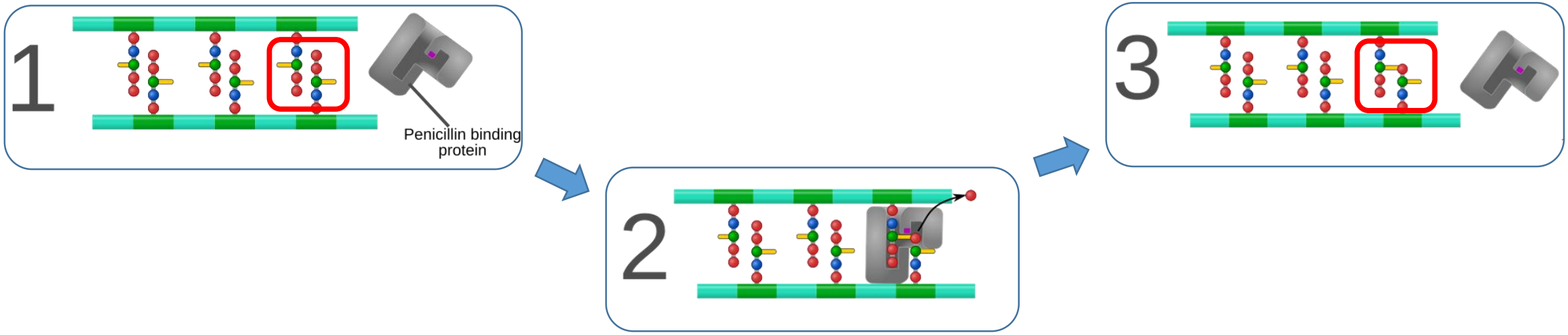
- Naturally resistant to betalactams
- fastidious – hard to culture

Mycoplasma pneumoniae - atypical pneumonia

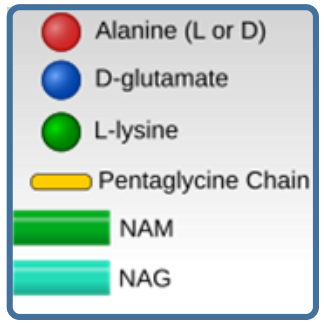
M. hominis and *M. genitalium* - urogenital infections

Cell wall synthesis - the target of antibiotics

Peptidoglycan filament linking by PBP activity - linking of side peptide bridges



Cross-linking Mechanism: Penicillin-Binding Proteins (PBPs) function as transpeptidases, creating the critical peptide bonds that turn loose polymer fibers into a rigid, net-like structure.



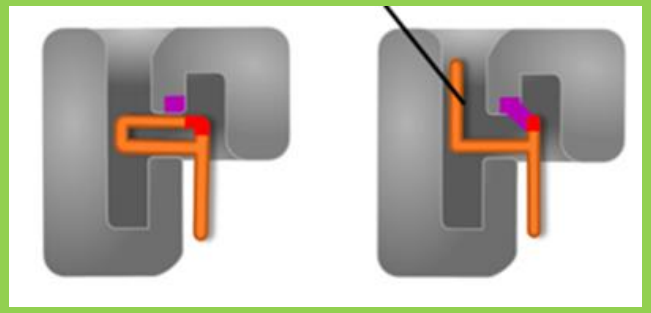
Images courtesy of Wikipedia

Cell wall synthesis - the target of antibiotics

Beta-lactams (penicillins and cephalosporins)

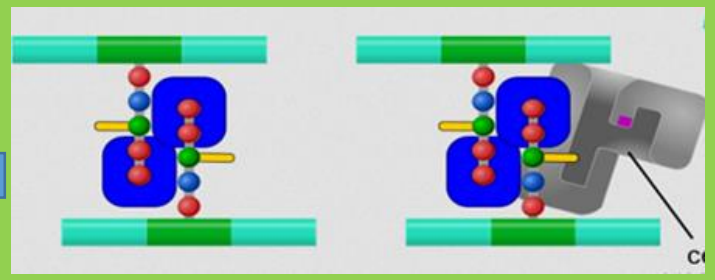
Glycopeptidy (vancomycin, teicoplanin)

Blocked catalytic site



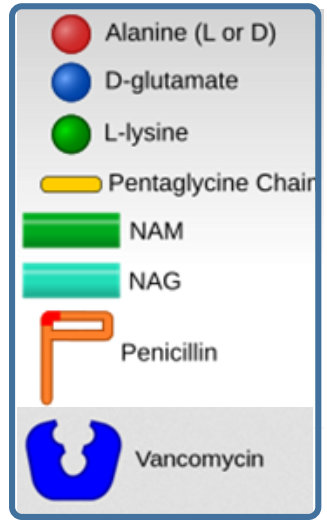
Mechanism of action

Blocking the PBP function



Blocking substrate recognition

cell lysis



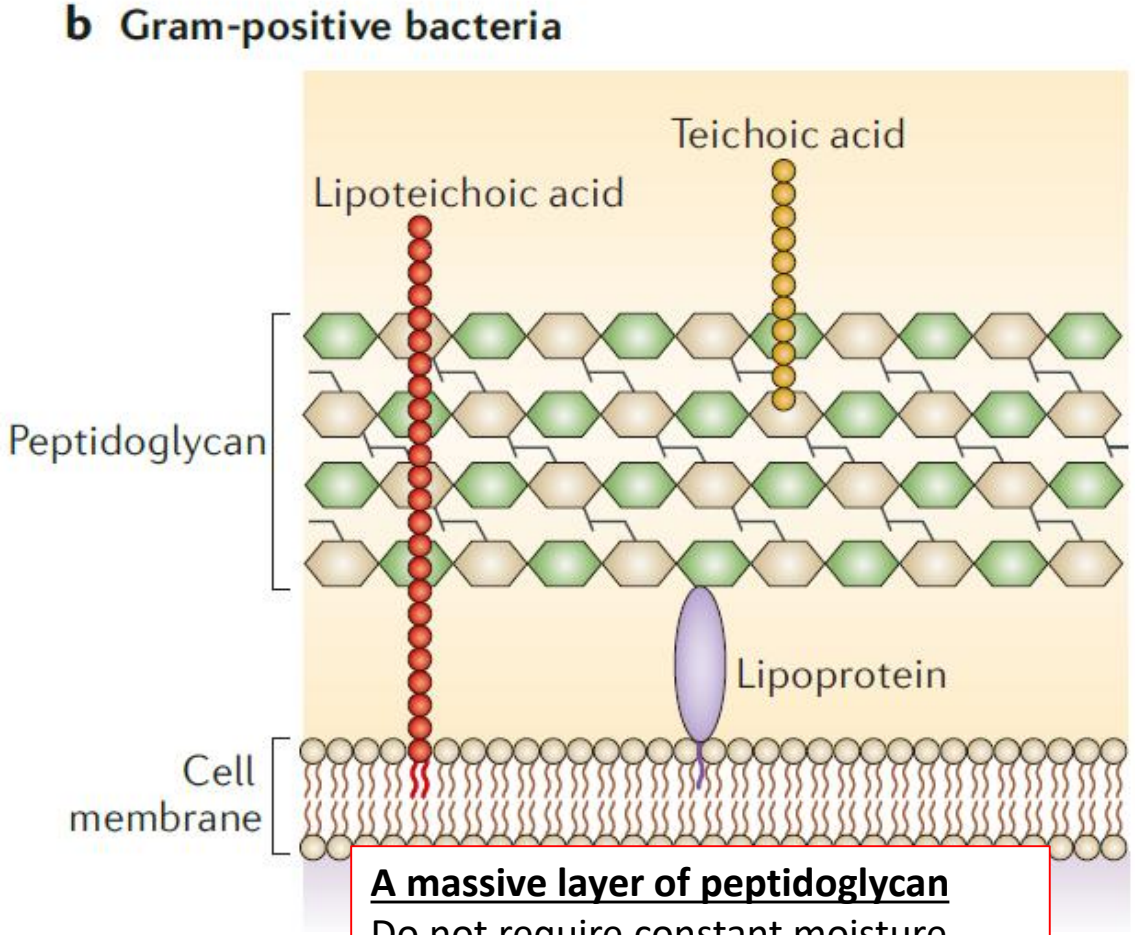
Resistance

lysis of antibiotic - betalactamase
change of PBP - PBP2a in MRSA

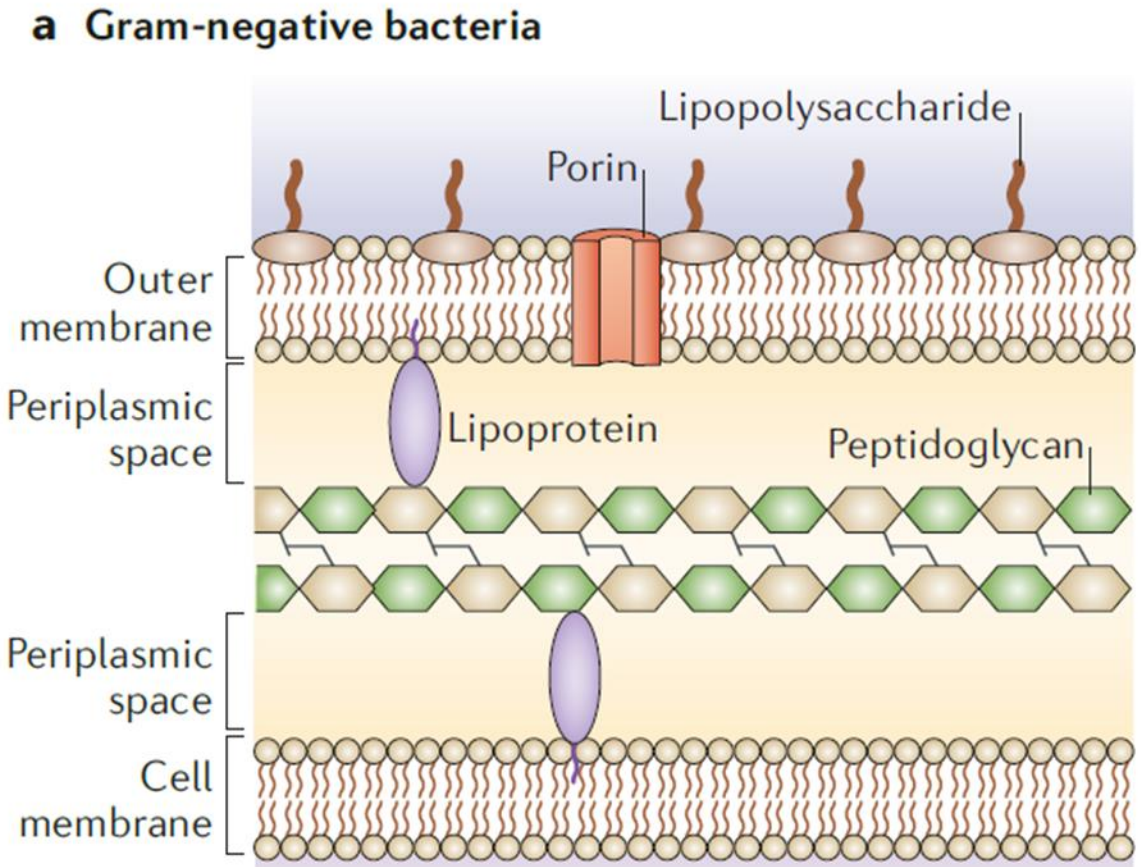
modification of the target site - *vanA/B* at VRE

Images courtesy of Wikipedia

Cell wall: gram positive vs negative



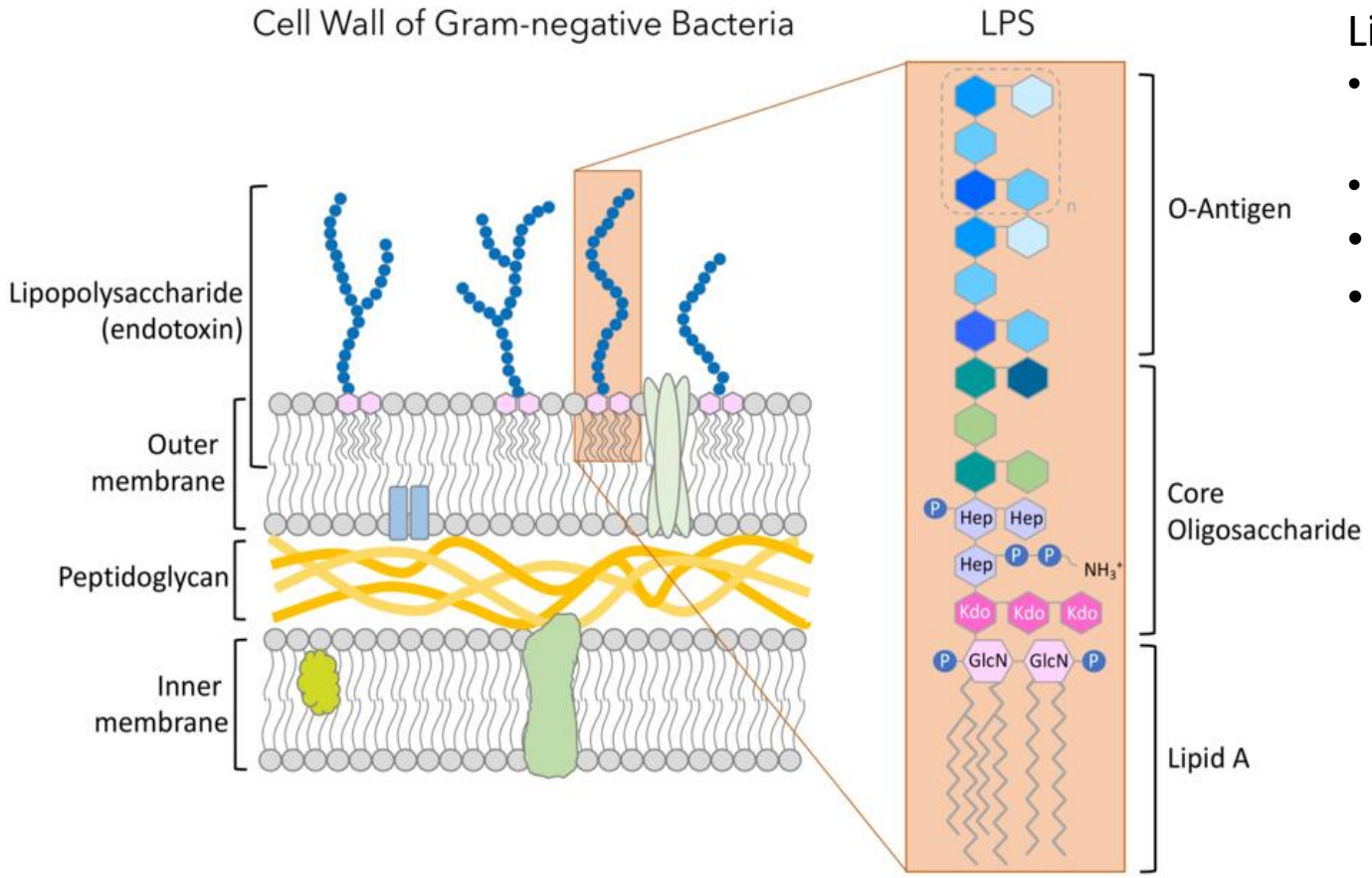
A massive layer of peptidoglycan
Do not require constant moisture
Physical resistance (drying, radiation)
Teichoic and lipoteichoic acid
- Adhesion
- Strong antigens (PAMPs)



Thin layer of peptidoglycan
Require moisture
Outer membrane - Lipopolysaccharide
Periplasmic space
Chemical resistance (antibiotics, enzymes, toxins)

Brown et al 2015 Nat Rev Microbiol

Cell wall: outer membrane of Gram-negative bacteria



Lipopolysacharide (LPS)

- O-antigen
  - Highly variable – e.g. serotyping *E. coli* **O157**:H7
- Core polysaccharide
- Lipid A – toxic part of LPS
- **Endotoxin** = part of the cell
  - could case septic shock
  - PAMP

Serotyping
How? - agglutination with antiserum (or DNA sequencing)
Why? - differentiation of *E. coli* strains with different pathogenic potential (EHEC, EPEC...)
- Epidemiology - spread

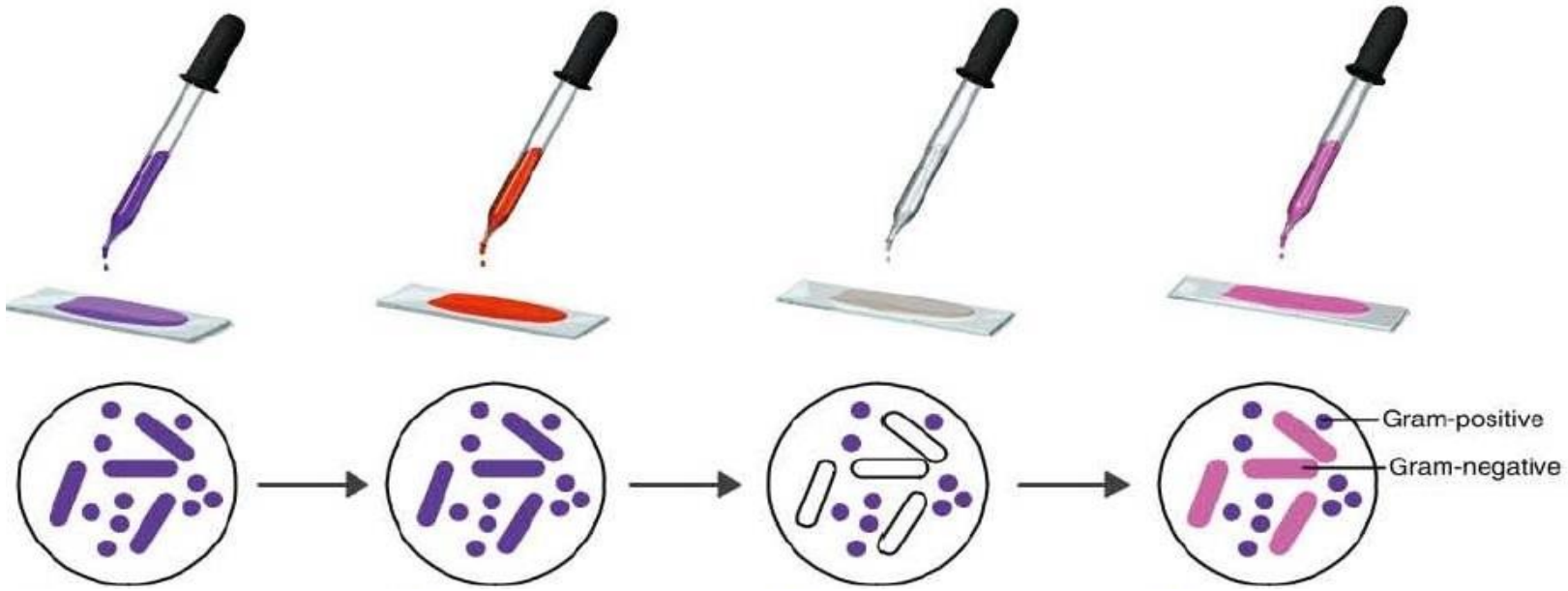
PAMP (**P**athogen-**A**ssociated **M**olecular **P**attern)
A molecule typical of bacteria that is recognized by non-specific (cellular) immunity (complement, macrophages)
- LPS, flagellar proteins, peptidoglycan....

Outer membrane as a target of antibiotics: polymyxins (colistin)

Gram staining – most important staining in microbiology

Allow to visualise and distinguish bacteria in clinical sample

Procedure:

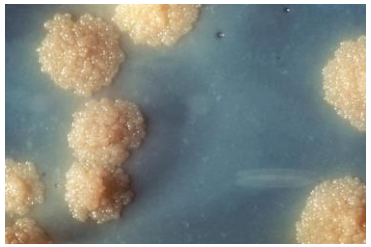


Violet **L**ugol **A**lcohol **K**arbolfuchsin

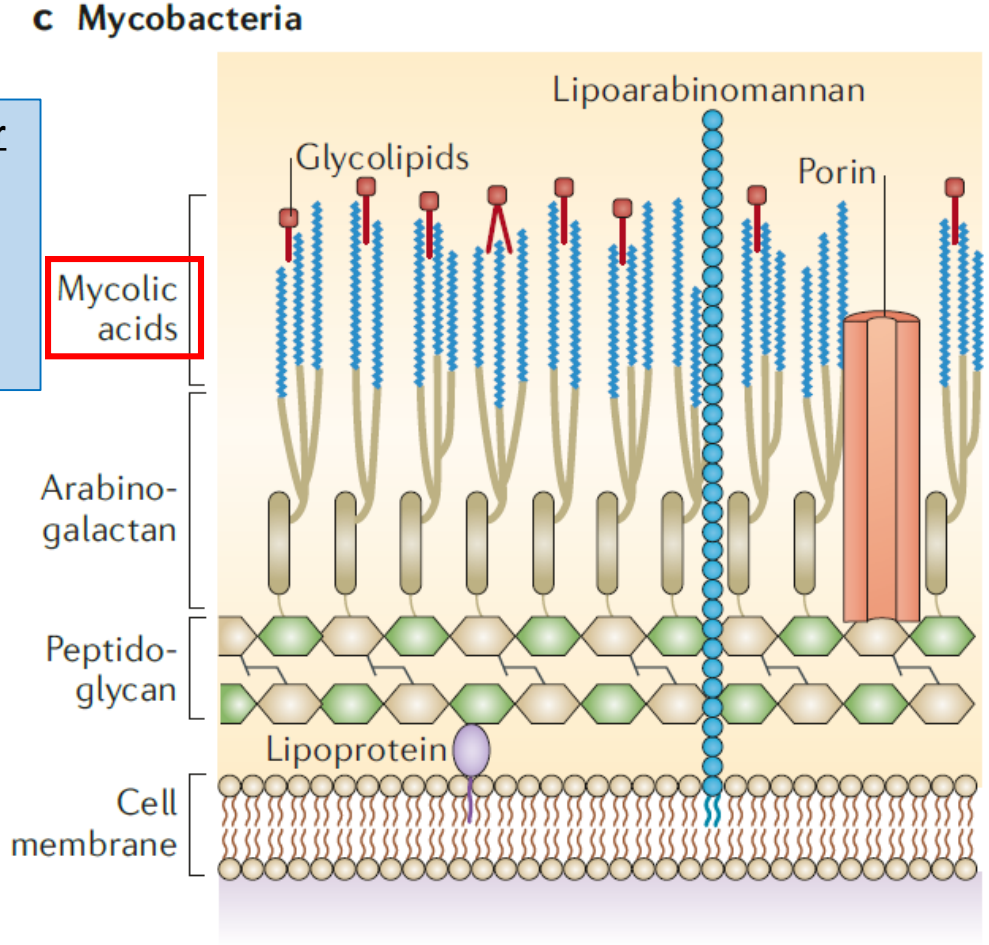
Vlak = train

Cell wall: Mycobacteria – Gram resistant bacteria

Mycobacteria are peculiar
M. tuberculosis
M. leprae
stains poorly
grow poorly
difficult to treat

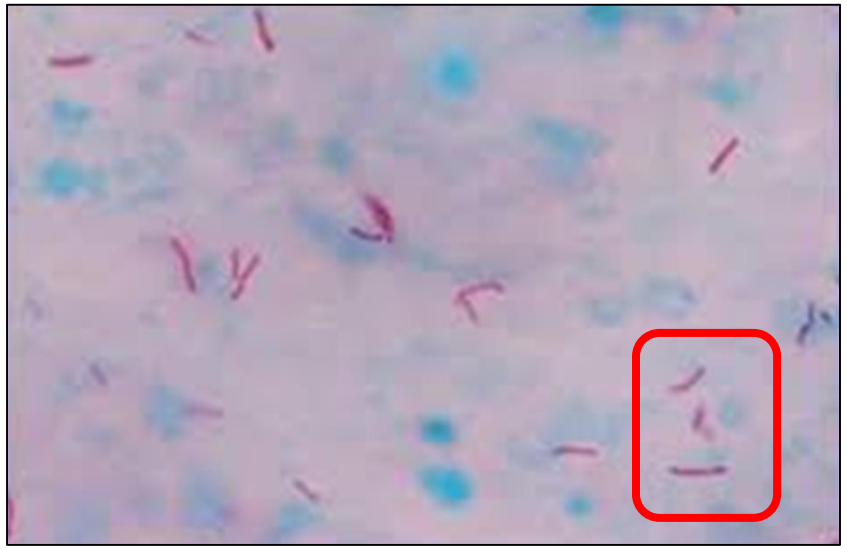


TB



Mycolic acids - waxy substances
- Repels both dye and alcohol - Gram does not work
- Physical and chemical resistance - ATB resistance

Acid fast (Ziehl-Nielsen) staining
- Specific for mycobacteria - TB diagnostics
- Mycobacteria pink everything else green



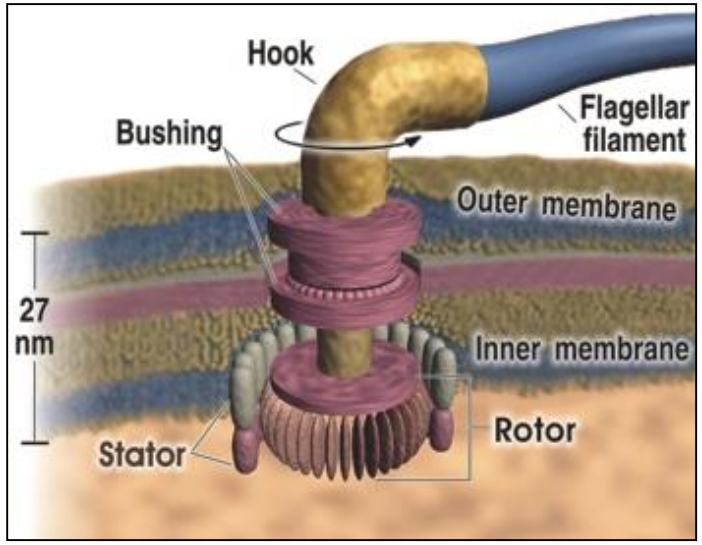
Q: How long does mycobacterium tuberculosis grow?

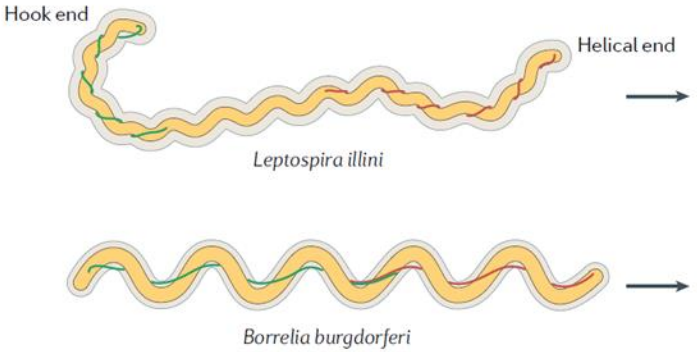
A: two weeks!

Surface structures: bacterial flagellum

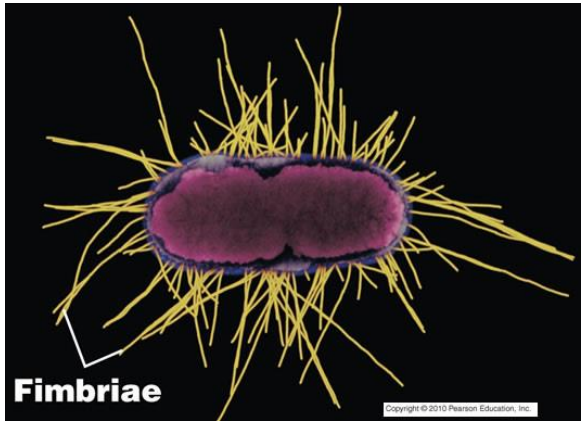
- **strongly antigenic (PAMP)**
  - Enterobacteriaceae serotyping: **H antigen**
- movement in a liquid environment - the propeller principle
- Who has (e.g. intestinal and environmental):
  - Most enterobacteria (*E. coli*, *Salmonella*, *Campylobacter*)
  - Others: *Pseudomonas*, *Bacillus*...
  - Advantages - movement in a liquid environment, nutrient seeking (chemotaxis), penetration of mucosal barrier and adhesion to epithelium
- They do not (e.g. skin bacteria)
  - streptococci, staphylococci, mycobacteria...
  - Why they don't need to - they have enough nutrients where they are, the flagellum irritates immunity (PAMPs) and is costly to form

- in spirochetes, the flagellum (=axial filament) passes through the entire cell and causes a rotational movement allowing tissue penetration (corkscrew)

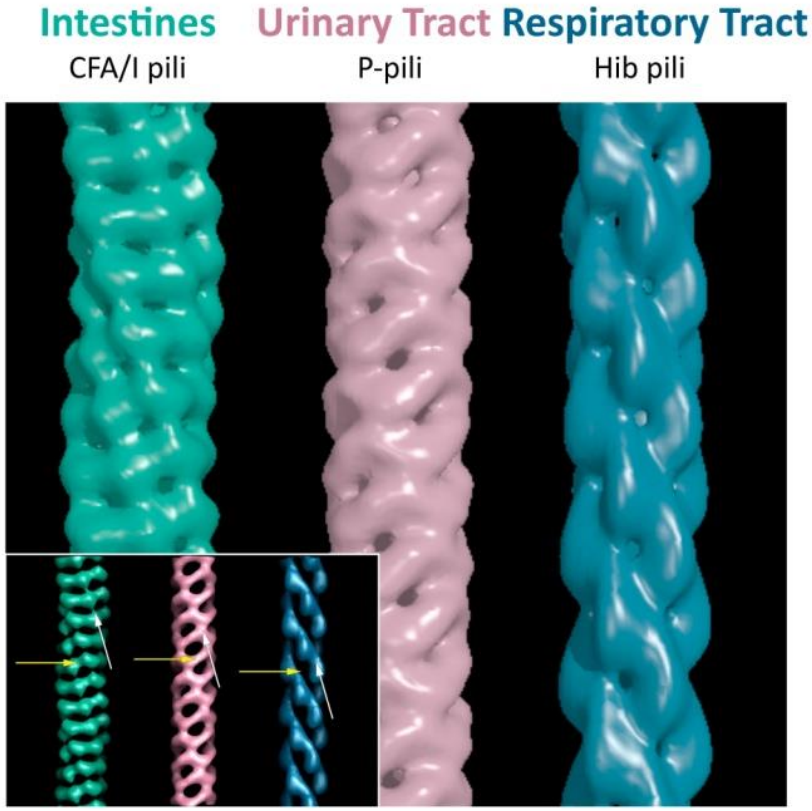




Surface structures: the Fimbria and Pili

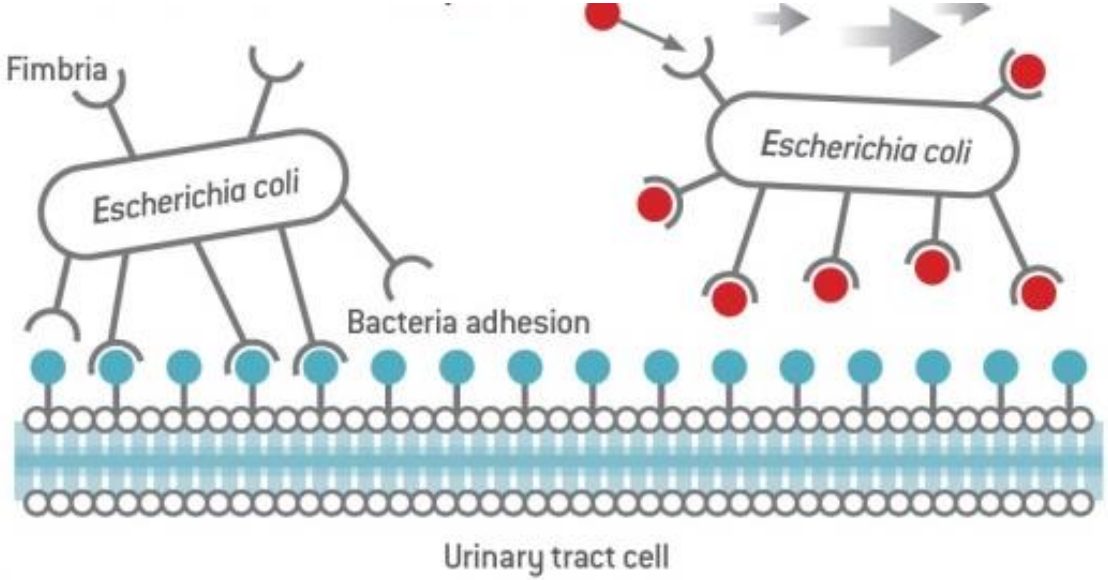


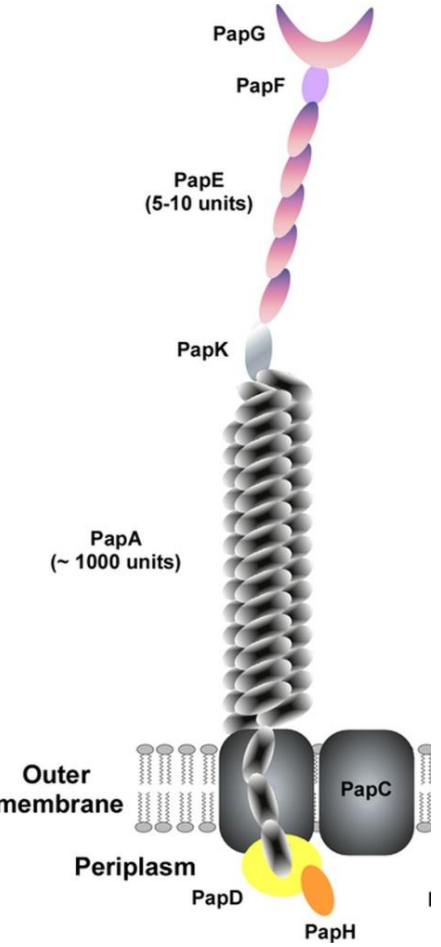
- synonym pili (pilus)
- Shorter than flagella, around the surface of the cell
- Made of proteins (like flagella)
- Sex pili – conjugation (plasmid transfer)
- **Main function is adhesion**
  - **adhesins** on the top of the pili
  - adhesin bind specific sacharide on the surface of human cells
  - **Tissue specific (i.e. UPEC –uropathogenic *E. coli*)**



Pili and *Escherichia coli* urinary tract infection

- PAP fimbriae (**P**yelonephritis-**A**ssociated **P**ili)
- Natural remedy: cranberries
- carbohydrates from cranberries are excreted in the urine and block adhesins (competitive displacement)





Structure of pili

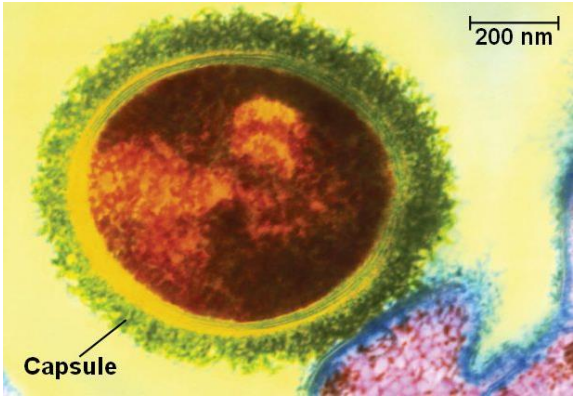
Surface Structure: Capsule

Capsule ≈ slime≈ glycocalyx

Additional protective cell layer in some bacteria

composed of polysaccharides

Halo around bacterial cells when stained by Burry stain

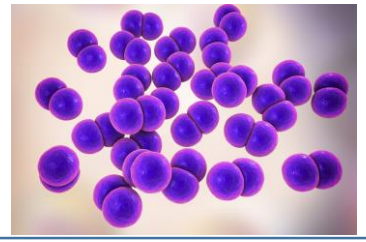


Electron microscopy

<u>What it is for:</u>

1. Helps in adhesion (sticks)
2. Protects bacteria from drying out (retains water)
3. And from the **<u>immune system</u>** (masks antigens - PAMPs, inhibits phagocytosis)
   - Key **virulence** factor
     - Non-encapsulated strains of *S. pneumoniae* are non-virulent. Lack of a polysaccharide capsule allows for rapid opsonization and phagocytosis by the host immune system.
   - High **antigenic variability** allows for immune evasion by hindering the adaptive (antibody) response.
   - K-antigen in enterobacteria
   - Target of vaccines (conjugated vaccines - capsule polysacharide antigen + protein carrier e.g. tetanus toxoid - better stimulates immune response)

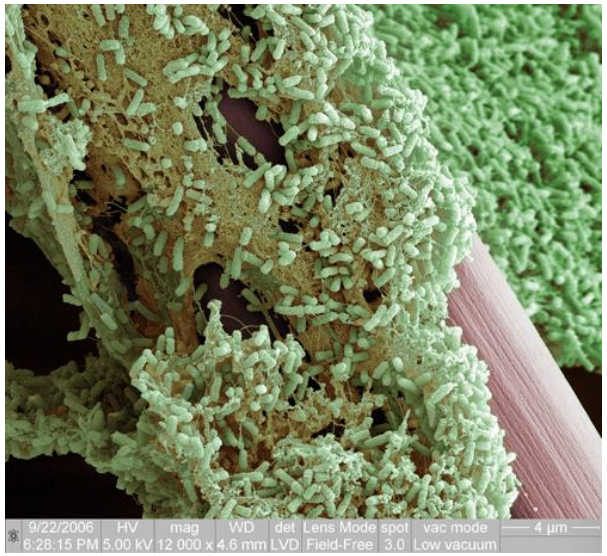
Important encapsulated bacteria

	Neisseria meningitidis (meningococcus)	*Streptococcus pneumoniae* (pneumococcus)	*Haemophilus influenzae* type B
Morphology:	G- (diplo)coccus	G+ (diplo)cocci in chains 	G- coccobacilli
Infection:	**meningitis**, sepsis...	**meningitis**, sepsis, pneumonia, otitis media...	**meningitis**, epiglottitis,...
Vaccinations:	Nimenrix (A,C,W,Y), Bexsero (B) recommended, covered Children, adolescents	Synflorix, Prevenar recommended, covered Children, seniors 65+	Part of the hexavaccine Required from 2 months of age

More encapsulated bacteria
Klebsiella pneumoniae
Bacillus anthracis
Salmonella Typhi
Streptococcus agalactiae

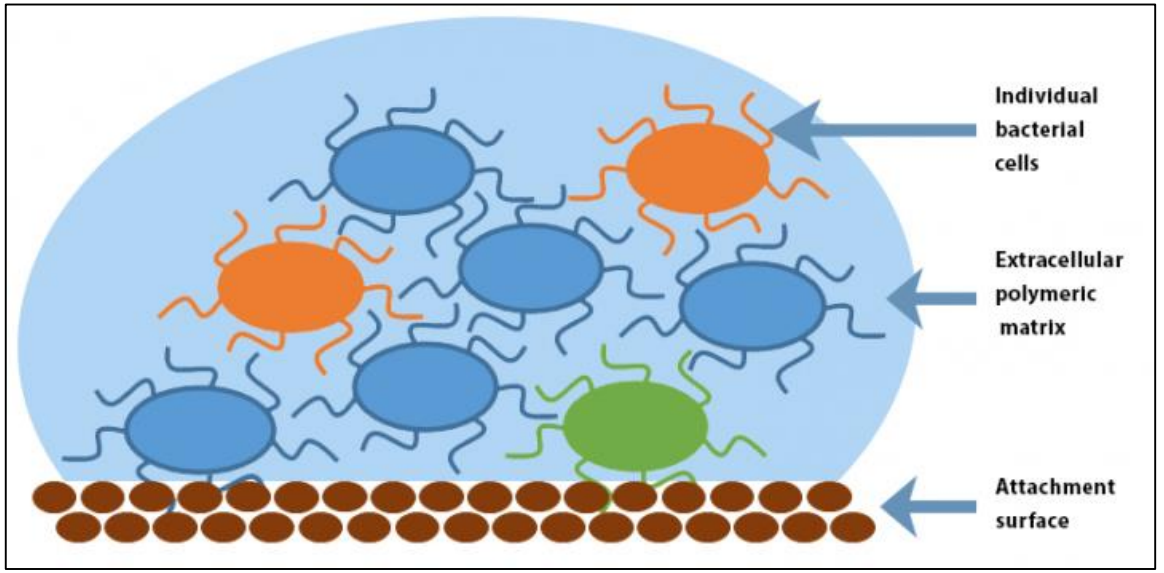
Biofilm

- Bacteria dont form a tissue
- But they could form a biofilm!!!
- Biofilm is **attached** structured consorcium of bacteria enveloped by **extracellular matrix** from polysacharides, proteins and extracellular DNA secreted by these bacteria



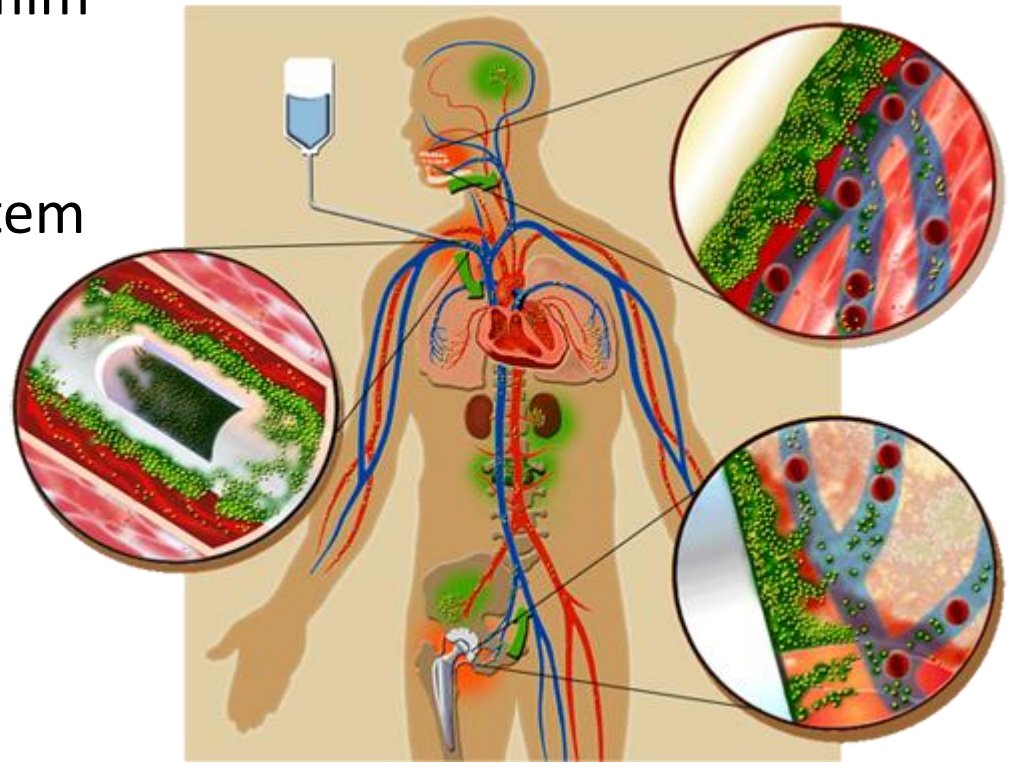
The polysaccharide layer is shared by the whole community (vs. the capsule belongs to a specific cell)

- Base of the biofilm is protected from
  - antibodies
  - complement
  - phagocytes
  - antibiotics
- Regeneration from the base

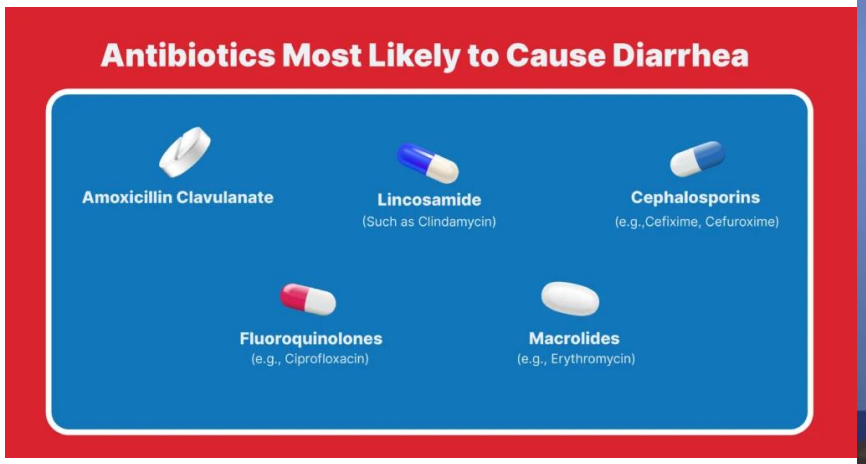


Biofilm – Clinical impact

- Majority of pathogenic bacteria forms biofilm
  - ***Staph. epidermidis***, *S. aureus, Pseudomonas aeruginosa, E. coli…*
- Protects from antibiotics and immune system
- Infection of foreign bodies:
  - **implants**
    - prostetics
    - dental implants
  - **catheters**
    - vascular
    - urinary
  - Dental plaque
- Biofilm is impossible to eradicate



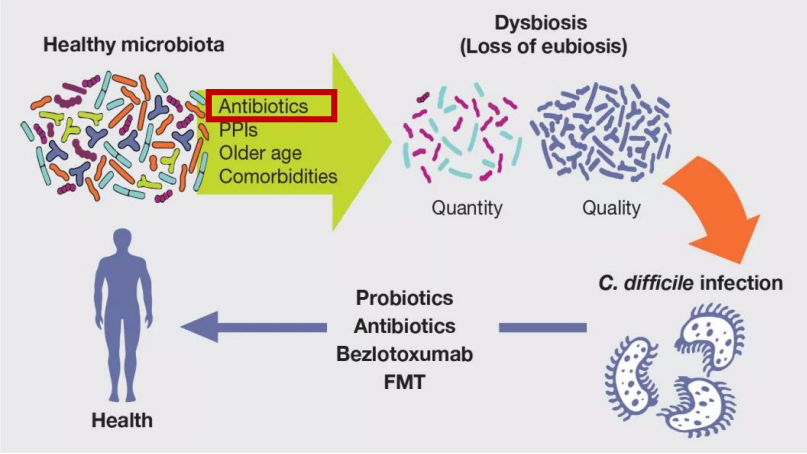
What have these picture in common?



Spore-forming bacteria!!!

Spore

What is it?	Resistant, resting stage
Importance	Surviving adverse conditions Transmission - long-term contamination Difficult to remove
Who	**G+:** *Bacillus* (anthrax), Clostridia (anaerobic bacteria): *C. tetanii* *C. botulinum* *C. difficile* (infection after ATB treatment)
	Intestinal and soil bacteria



Spore survives

Drying

boiling

UV

alcohol disinfection

Spore will destroy

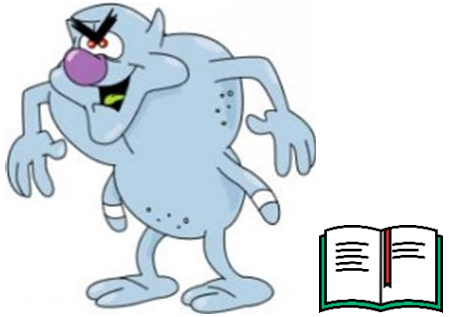
autoclave
121 °C, 15 minutes, 1.1 bar

Sporicidal disinfection
Active ingredients: hydrogen peroxide, peracetic acid, glutaraldehyde, hypochlorites

Attention mushroom spore is something else!

Bacterial pathogenesis

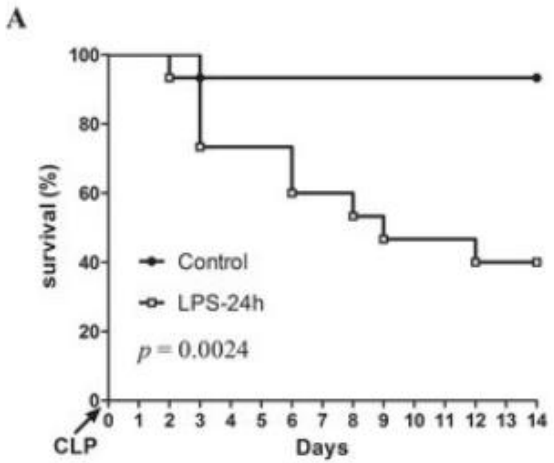
Why are bacteria 'bad'? (Understanding Pathogenicity)



- **Pathogenicity** = The ability of a microorganism to cause disease
  - **Qualitative Trait:** Expressed as a binary (**Yes/No**)
  - Refers to a specific **bacterial species**
- **Virulence** = The **quantitative** measure of pathogenicity
  - Refers to a specific **bacterial strain**
  - *Example:* Commensal *E. coli* strains vs. virulent pathotypes (EHEC, EPEC, etc.)
- **Lethal Dose LD_{50} –** The number of bacteria required to kill 50% of the exposed population
- **Infectious Dose ID_{50}** - The number of bacteria required to cause disease in 50% of the exposed population

$$Infection = \frac{Virulence \times Dose}{Host\ Resistance}$$

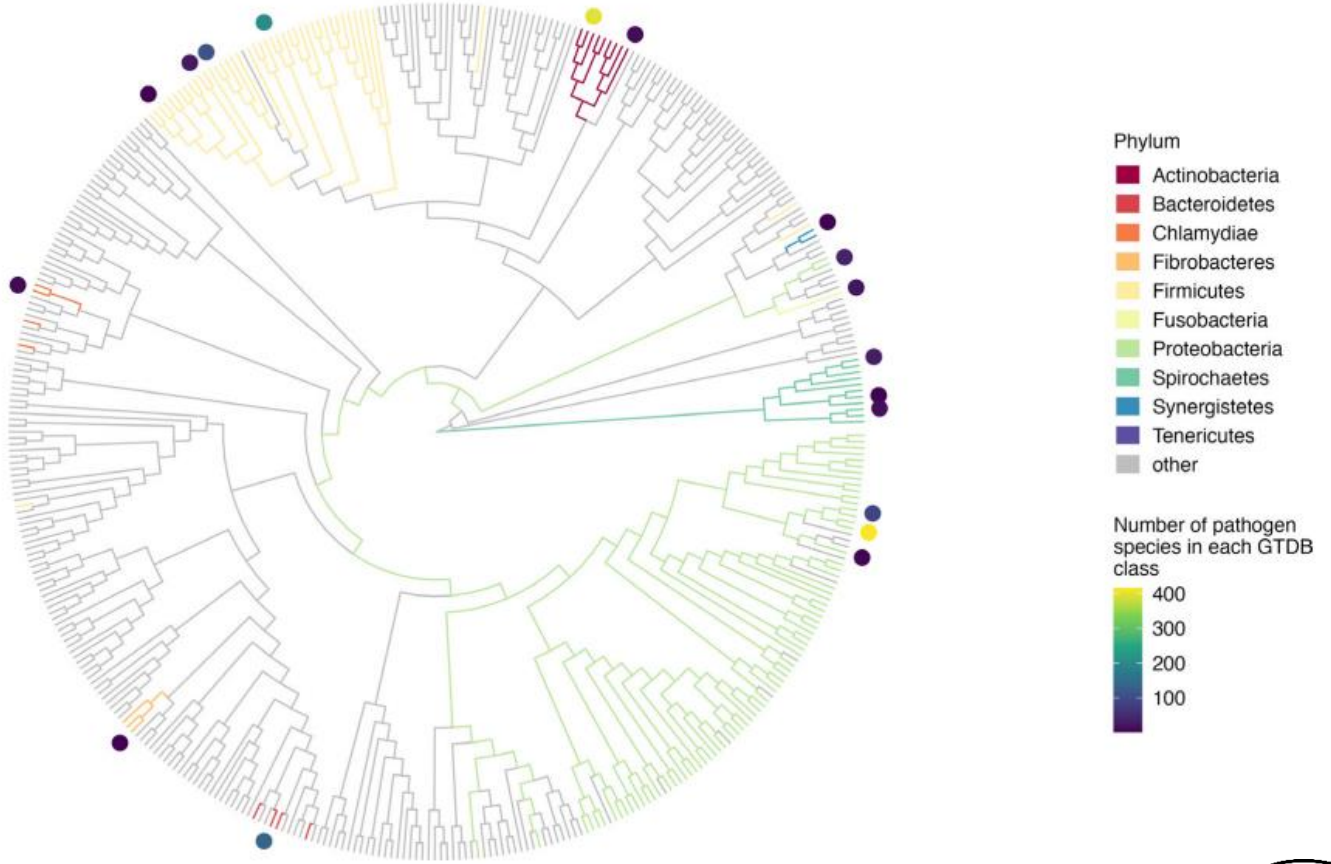
ID and LD testing:



No.	Bacteria	Symptoms onset	Infectious dose (CFU/mL)
1	*Listeria monocytogenes*	A few days to 3 weeks	$400–10^3$
2	*Salmonella*	12 to 24 h	$10^4–10^7$
3	*E. coli*	12 h to 3 days	$10^1–10^2$
4	*Campylobacter jejuni*	2 to 5 days	400–500
5	*Listeria monocytogenes*	2 days to 3 weeks	<1000
6	*Staphylococcus*	1–7 h	$>10^6$
7	*Shigella*	12–50 h	<10
8	*Bacillus cereus*	30 min to 15 h	$>10^6$
9	*Clostridium botulinum*	12–36 h	<10
10	*Clostridium perfringens*	8–22 h	$>10^8$
11	*Yersinia enterocolitica*	-	1–3 days
12	*Vibrio parahaemolyticus*	4 h–4 days	$>10^6$

How many bacteria are pathogenic?

- 1,531 species of pathogenic bacteria ≈ 7% of all bacterial species
- (But a very benevolent definition of pathogen = at least 3 publications documenting infection with the agent)



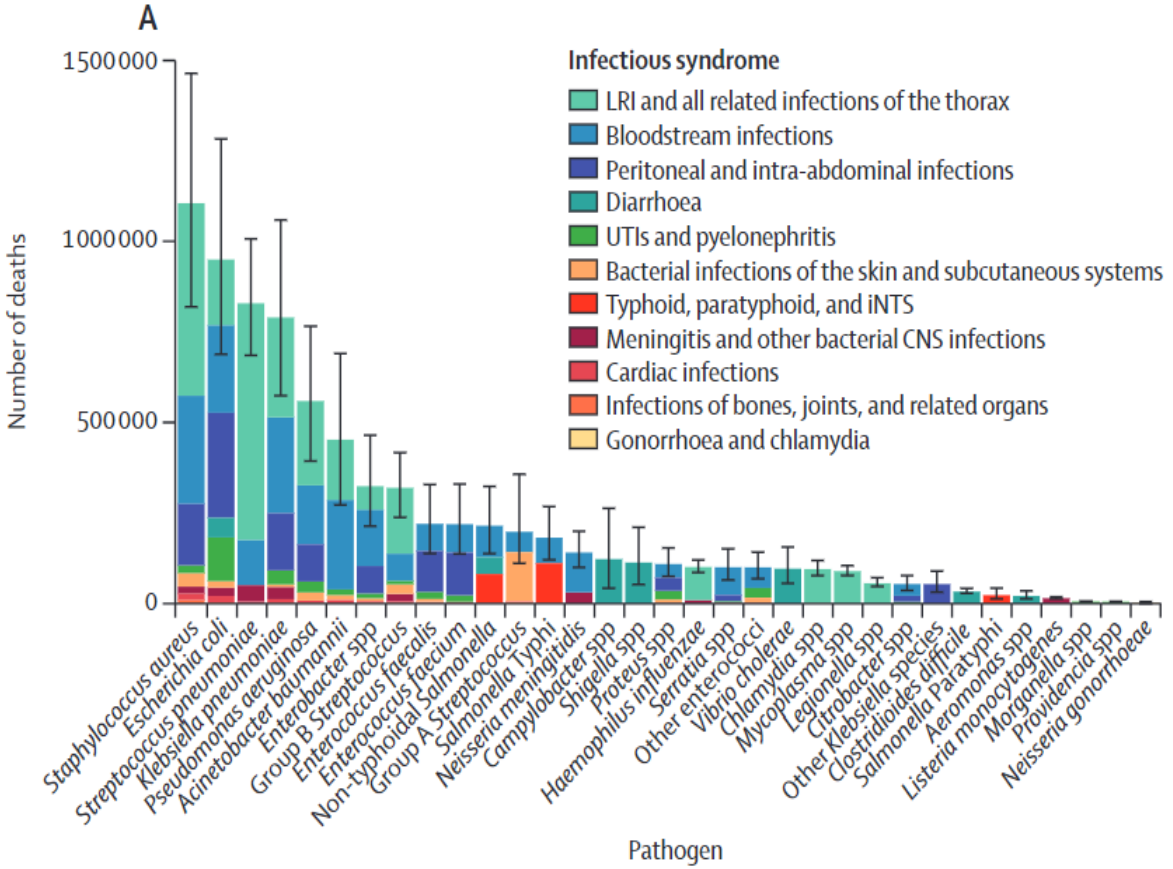
The real pathogens are even fewer...

Only 33 species of bacteria
>50% of deaths from bacterial infections

- (7.7/13.7 million deaths/year)

(note: the study does not include TB≈1.6 million deaths in 2021)

Top:
Staphylococcus aureus
Escherichia coli
Streptococcus pneumoniae
Klebsiella pneumoniae
Pseudomonas aeruginosa



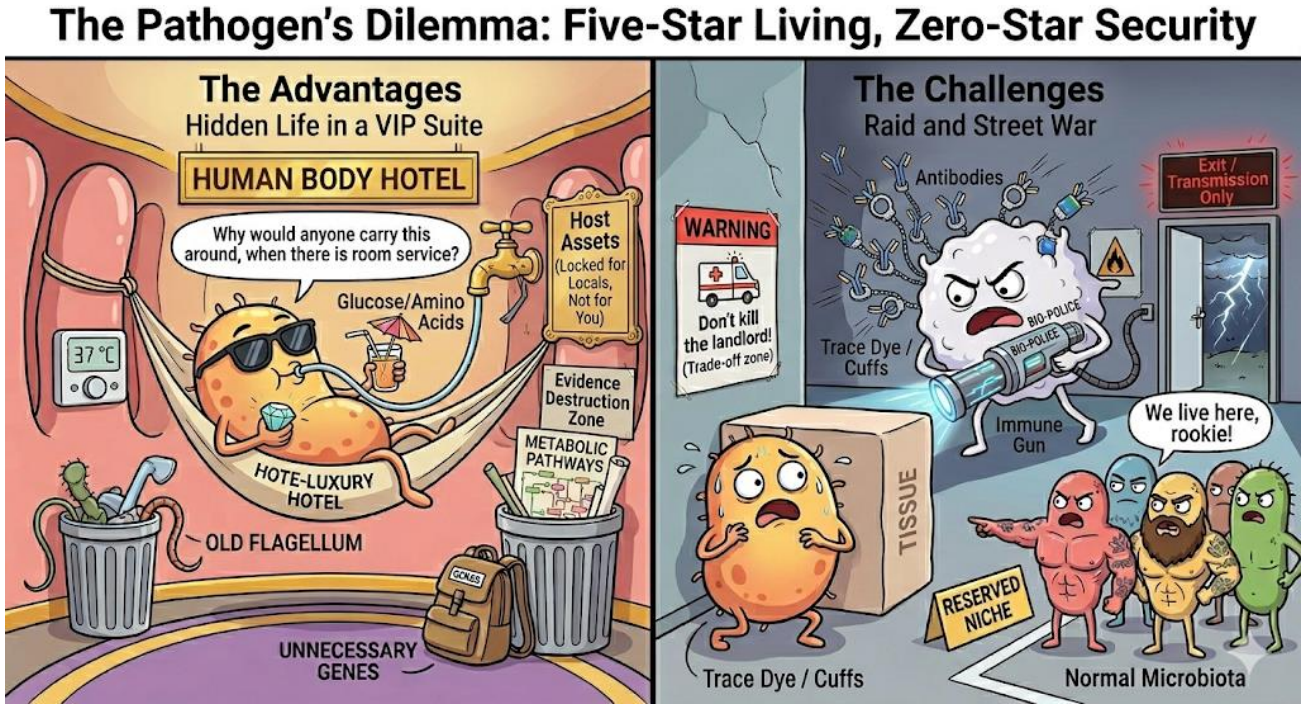
Why are bacteria pathogenic?

The Advantages: The Human Body as a "Premium" Habitat

- **Homeostasis:** Provides highly stable conditions (consistent temperature and pH).
- **Abundant Resources:** Continuous supply of nutrients and moisture.
- **Genome Streamlining:** Parasitic/pathogenic bacteria often undergo **Genome Reduction**.
  - *Loss of redundant genes:* If the host provides nutrients, the bacteria can discard complex metabolic or motility genes (e.g., flagella or biosynthetic pathways).

The Challenges: The Cost of Pathogenicity

- **Host Immunity:** The primary barrier; constant pressure from innate and adaptive defenses.
- **Niche Competition:** High "competence" requirements—competing with the resident **Microbiota** for space and resources.
- **Host Viability:** A pathogen must balance damage; killing the host too quickly terminates the bacteria's "home."
- **Transmission Bottlenecks:** The challenge of moving from one host to the next before the current host dies or clears the infection.



Breaking into the host, stealing resources, and evading the immune 'police'—**pathogenesis is a life of criminal**

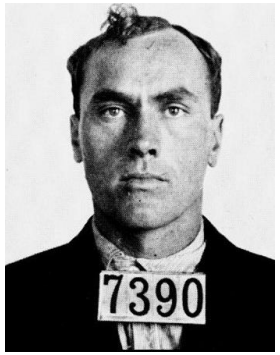
Commensal vs pathogen

stable growth	rapid growth
does not harm the host	host exploitation
transmission accidental	infection helps spread

Pathogen Typology: The Criminal Profiles

Obligate pathogen:

The Born Predator



type Carl Panzram

„I get a kick out of murdering people.“

"In my lifetime I have murdered 21 human beings, I have committed thousands of burglaries, robberies, larcenies, arsons and, sodomy on more than 1,000 male human beings. For all these things ***I am not in the least bit sorry***. I hate the whole damed human race including myself....I have no desire whatever to reform myself. My only desire is to reform other people who try to reform me. and I believe that the only way to reform people is to kill em. **My motto is**, *Rob em all, Rape em all and Kill em all*.“

Characteristic	Profile: Carl Panzram (Predator)	Profile: Obligate Pathogen (e.g., M. tuberculosis)
Mode of Existence	Life defined by violence. Incapable of peaceful coexistence within a community.	**Obligate parasite**. Incapable of living or reproducing outside the host (often has a reduced genome). Any clinical finding is **always considered pathological.**
Attack Trigger	Unprovoked attack. Does not require weakness or a mistake from the victim; their mere presence is enough.	**High virulence. Causes disease even in completely healthy individuals**. Does not require a compromised immune system.
Biological Weaponry	Psychology and skills of a professional killer. Experience combined with brute force.	Specialized virulence factors. Toxins, enzymes, and immune evasion systems. The attack comes from the outside (**exogenous**).
Objective	Nihilism and destruction. The goal is to destroy the world and themselves with it.	Aggressive spread. The goal is rapid replication and transmission, even at the cost of severe host damage (high lethality). Disease is necessary for transmission between hosts.

Examples:

Bacillus anthracis (Anthrax), *Treponema pallidum* (Syphilis), *Yersinia pestis* (Plague)…

Extremely rare but extremely dangerous

Pathogen Typology: The Criminal Profiles

Opportunistic Pathogen:
The friendly neighbor

type: John Wayne Gacy, „the Killer Clown“

"You know ... clowns can get away with murder."

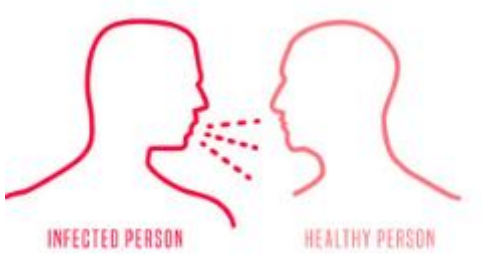
„The idea that I'm a homosexual thrill killer, that I stroll down the streets and stalk young boys and slaughter them... Hell, if you could see my schedule, my work schedule, you knew damn well that I was never out there.“

Characteristic	**Profile: John Wayne Gacy**	**Profile: Opportunistic Pathogen (e.g., *Staphylococcus aureus*)**
Social Role	Pillar of the community. Successful contractor, politically active, clown for sick children.	**Common microbiota**. Lives on the skin or in the nose of 30% of people. It’s "part of the team."
"Usefulness"	Benefit to the surroundings. Organized neighborhood parties, gave people jobs.	Barrier protection. Occupies space on the mucosa to prevent other (worse) aggressors from invading.
When it turns into a monster?	Absence of control. Exploited the trust of young people and the failure of authorities' oversight.	**Immune failure**. Exploits a wound (surgery), diabetes, or weakened immunity (HIV, medication).
Modus Operandi	Invited the victim to his house (into "his own territory").	Does not attack from the outside; you have it within you (**endogenous**).
The Result	Shocking destruction. No one expected that the nice man had a graveyard under his floor.	A common skin commensal is one of the deadliest bacteria.

Examples:
Staphylococcus aureus, *Escherichia coli*, *Clostridium difficile*, and many more

The pitfalls of a pathogenic way of life

<u>The cycle of infection:</u>



1. transmission

2. infection

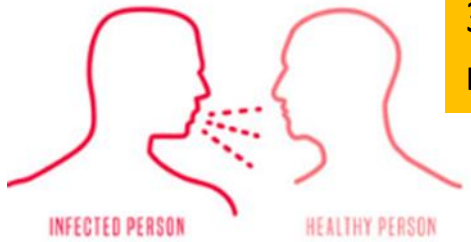
Defence against IS
Propagation - nutrient exploitation

Host dies too soon

↑virulence

The host will recover too soon

↓virulence



3. Successful infection of the new host

Virulence of the bacterium

Viability of the host

Long-term interaction leads to optimized virulence

What allows high virulence

Host:

High transmission rate

- Large and dense population
- Frequent connections between populations and within
- Sensitive people

High virulence = rapid serious disease with high mortality

Bacteria:

Infection of wrong host (new host, or there is reservoir in another host)

- *Yersinia pestis*

Could survive without host– e.g. spores, environmental reservoir

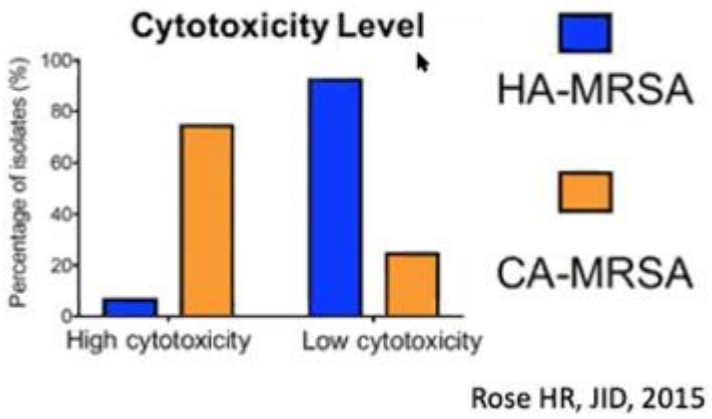
- *Bacillus anthracis*
- *Clostridium tetanii* and *Clostridium botulinum*
- *Legionella pneumophila*

Infection in wrong localisation

Staphylococcus aureus:

- colonisation x localised skin infection x systemic infection

The paradox of MRSA nosocomial pneumonia mortality



Rose HR, JID, 2015

HA-MRSA **hospital** strains
CA-MRSA **community** strains

Feature	CA-MRSA (The Street Thug)	HA-MRSA (The Patient Burglar)
Territory	Gyms, schools, prisons.	ICUs, surgery wards.
Weaponry	High levels of toxins (e.g., PVL) that rip through healthy tissue.	Low toxin levels, but multi-drug resistance
Target Victim	The Athlete. Healthy people with strong immune systems.	The Invalid (catheters, ventilators, surgery wounds).
Modus Operandi	Smash and Grab. Fast, violent skin infections or necrotizing pneumonia.	Slow Infiltration. Waits for oportunity to cause deep-seated infection.
Body Count	Lower (victims fight back better).	Much Higher (victims are already defenseless).

HA-MRSA are **less** virulent, but hospital-acquired pneumonia caused by HA-MRSA has a much **higher** mortality rate.

The explanation?

Healthy people don't usually lie in hospitals!
A pathogen is only as deadly as the host is vulnerable

Virulence factors - what allows to cause an infection

- Virulence factors - basic breakdown:

1. Adhesins and colonization factors
2. Invasiveness factors
3. Secretion systems
4. Toxins
5. Immunity-interfering factors
6. (nutritional factors - e.g. iron acquisition)
7. (Biofilm)

Invasiveness

Invasiveness

- **Adhesion** - adherence to epithelia (adhesins, lipoteichoic acid, sheath)
- **Invasion** - penetration of the epithelium or into the host. cells
  - Enzymes - degradation of extracel. matrix - hyaluronidase, collagenase, elastase
  - Invasins - phagocytosis by non-professional phagocytes (e.g. epithelia)
  - flagella - penetration through mucus to epithelium or even through epithelium - spirochetes (corkscrew)
- **Secretion systems** - inject effector molecules into host cells
- **Defensive factors**: Capsule, biofilm

Toxicity

- Damage by direct action or through immune reaction
  - Endotoxins (already discussed – PAMPs)
  - Exotoxins
  - Bacterial metabolites
- Intoxication can take place even without the presence of the bacteria in the body!

Exotoxins

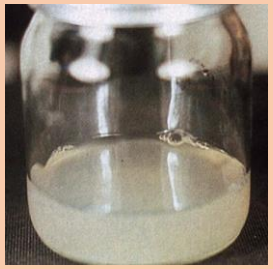
- = „true“ toxins
- Extracelullar **protein** moleculles (compare to endotoxin – outer membrane G-)
- **Direct and serious damage to host**
- Diferentiation – variable criteria:
  - chemical structure (single molecule or macromolecullar complex)
  - Targett structure (cell surface or intracellular)
  - Mechanism (**neurotoxic, enterotoxic**, **cytotoxic**)

Exotoxiny

- = "classic" toxins
- Secreted extracellular proteins that damage the host
- Frequently encoded by plasmids or phages
- By mechanism and site of action (may overlap):
  - **Generally cytotoxic (cytolysins)**
  - **Neurotoxic**
  - **Enterotoxic**
  - **Superantigens**

According to the structure, e.g. AB toxins
Two subunits A (action) and B (binding)
Toxins inhibiting proteosynthesis
Hyperactivating toxins
Neurotoxins

Examples of diseases with a key role of the toxin:



Cholera
Vibrio cholerae

Whooping cough
Bordetela pertusis

Tetanus
C. tetanii

Botulism
C. botulinum

Necrotizing pneumonia
Staph aureus PVL+

Diphtheria
Corynebacterium diphtheriae

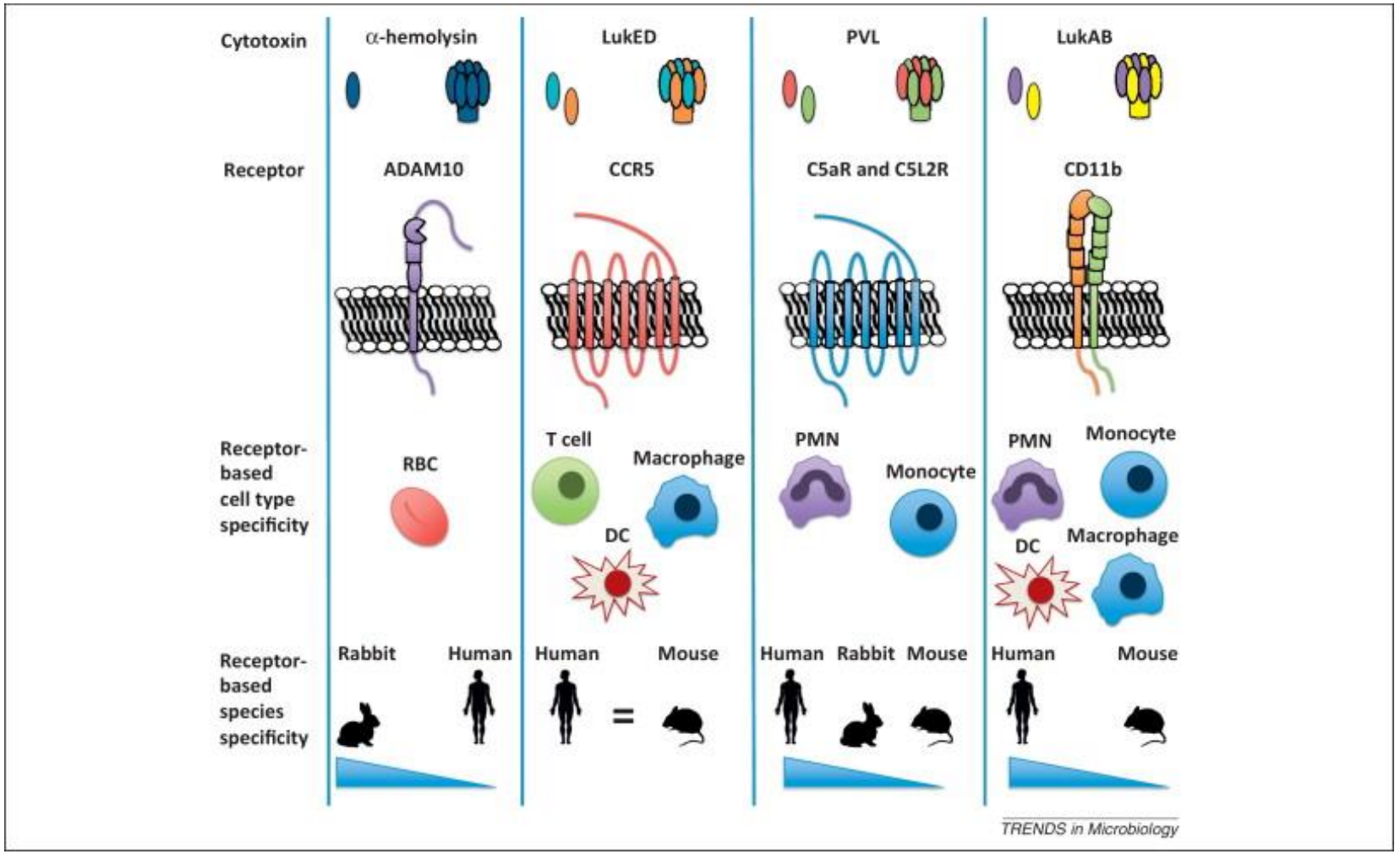
Food poisoning
Staph aureus
Bacillus cereus

Cytotoxins

Pore forming toxins
Mechanism of action

1. Subunits recognise receptor
2. Polymerisation of subunits
3. Insertion of the pore into the mebrane
4. Ion leakage
5. Cell lysis

Cytotoxins of *S. aureus*



lukED, luk AB, PVL - leukocidins

Neurotoxins

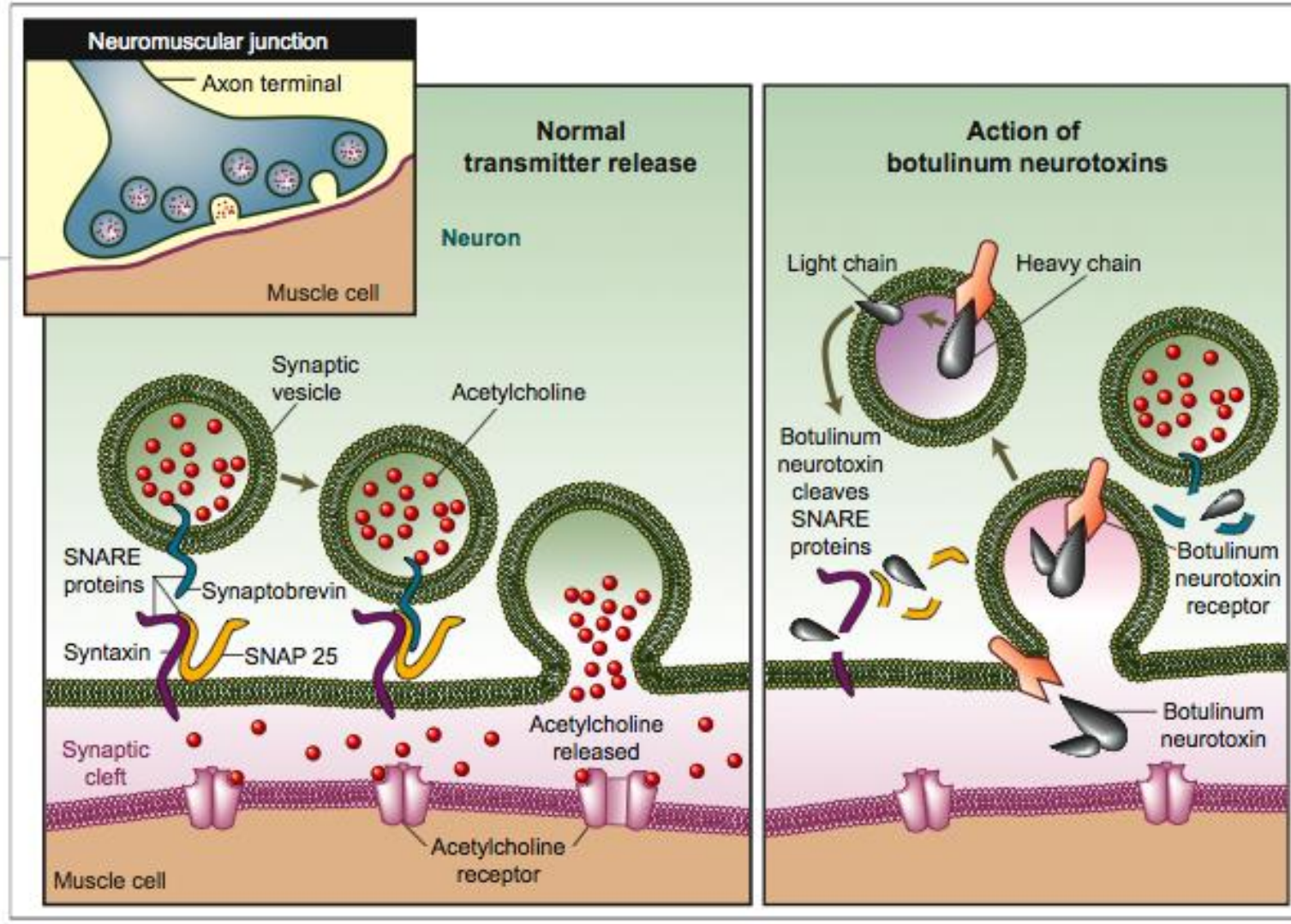
- extremely low lethal dose
- produced by Clostridia
  - sporulating
  - anaerobic

- mechanism of action: inhibition of synaptic vesicle and presynaptic membrane fusion

  - Botulinum toxin: inhibits acetylcholine release → muscle relaxation

  - Tetanospasmin: inhibits GABA and glycine release → muscle contraction

  - **disruption of the respiratory cycle**



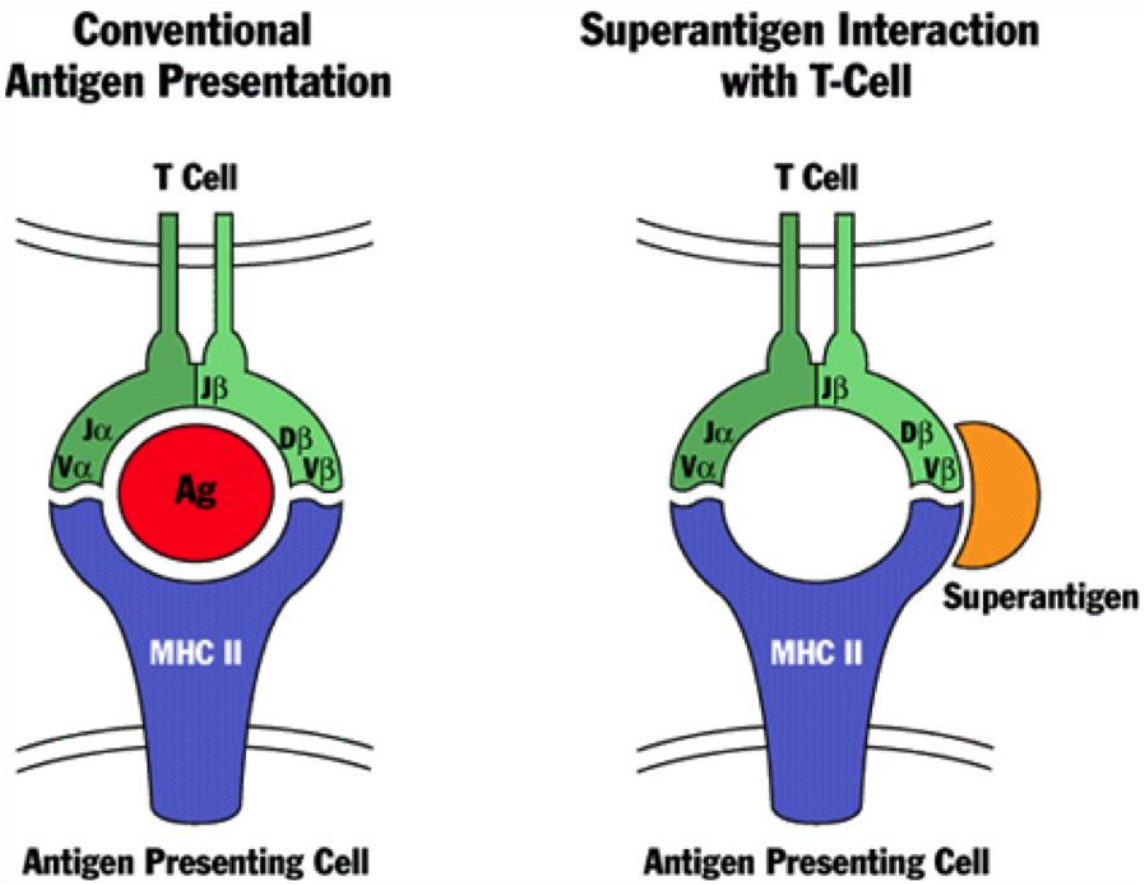
Enterotoxins

- Many patogenic bacteria
  - *E. coli, Shigella, Salmonella, Vibrio cholerae, Campylobacter, Clostridium difficile, S. aureus*
- Poissoning of the *intestinal* epithelium→ **diarrhoea**
  - Typical symptom of intestinal infection
- Diarrhoea
  - patogen: rapid spread in host population
    - Lots of stool filled with bacteria
  - host: cleaning of the intestines

Superantigens

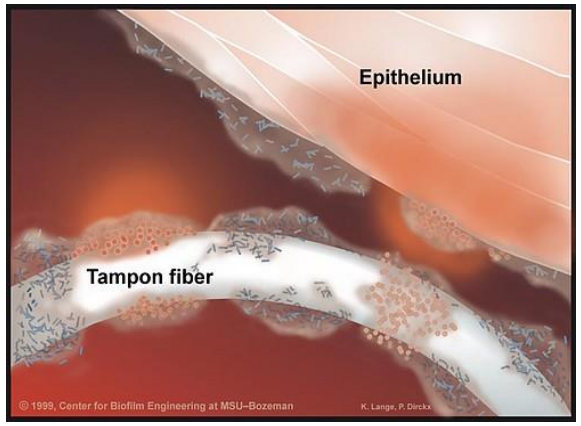
- **super**antigens
  - Direct cross connection between MHC II and TCR
  - **Without specific antigen**
  - **Masive activation of T-lymphocytes**

Examples:
Pyrogenic exotoxins of *Streptococcus pyogenes*
Toxin shock syndrome toxin of *Staphylococcus aureus*
Staphylococcal enterotoxins
Food poissoning



Toxic shock

- Caused by superantigens
- TSST-1 of *S. aureus* (less frequently caused by *S. pyogenes*)
- Similar to septic shock
    - Leads to massive cytokine production by activated T-lymfocytes
    - Fever, headache, rash, nauzea diarhoea
    - **Colaps of immune and regulatory homeostasis**
    - **Systemic patological changes**
    - Desquamation after infection
    - CZ 1983-2011 - 159 cases, (47 menstrual form). letality 11 %(staphylococci), 50% (streptococci)

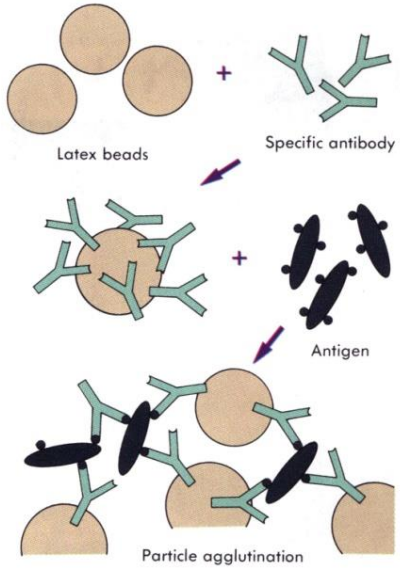


biofilm on tampon fibres reservoir of staphylococci producing toxic shock toxin

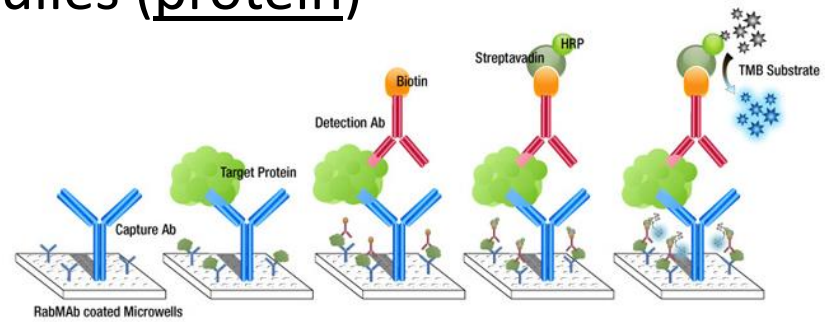
Toxin detection

Why?

Bacteria may not be present at all in the disease (e.g. enterotoxins)
Toxin is key to infection and may not be present in all strains (*C. difficile*)
Need to confirm whether the isolated strain produces toxin (PVL toxin in *S. aureus*)



- Detection of toxin moleculles (<u>protein</u>)
  - Specific antibodies
    - latex bead agglutination
    - ELISA
    - Rapid antigen tests



- Detection of toxin genes(<u>DNA</u>)
  - PCR specific for selected genes (p

Virulence regulation

Colonization

Polymicrobial
Low quantity
(stable)

Infection

Monomicrobial
High quantity
(exponential expansion)

Quorum sensing

expression of virulence factors is controlled by population density

<u>The Signal for a Riot:</u> Quorum sensing – the population density dependent cycle of destruction and spread

Low density, high adhesion, waiting for the first round

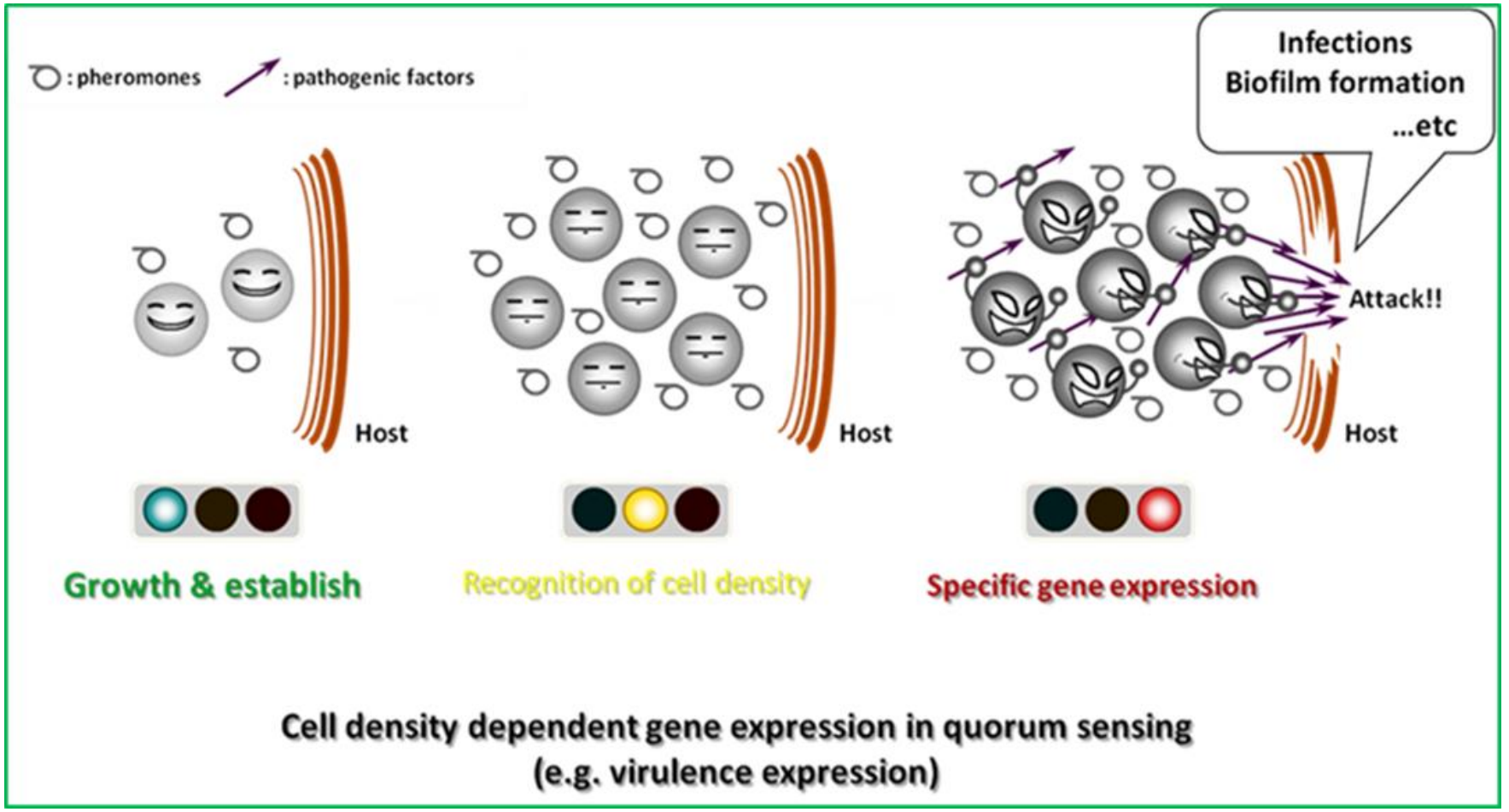
Surplus of nutrients ensured: growth and multiplication

Critical threshold reached, signal-receptor binding, positive feedback loop (Autoinduction).

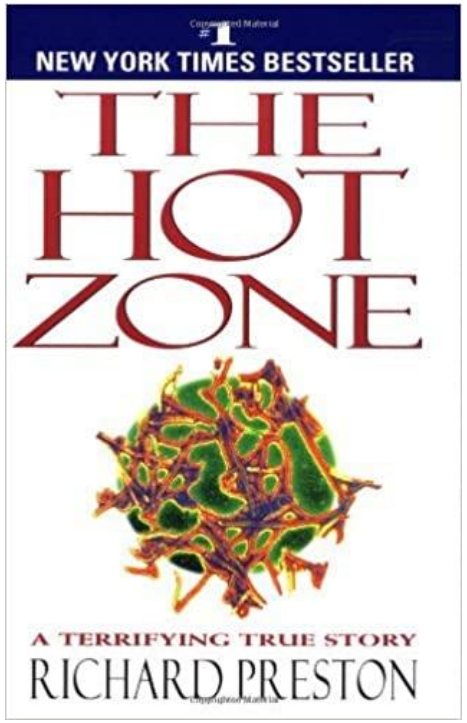
Synchronized shift in gene expression, massive production of virulence factors and toxins

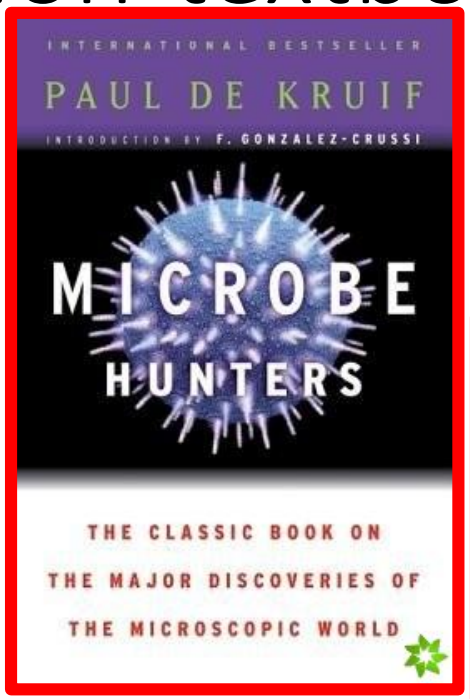
Tissue destruction, nutrient depletion, biofilm dissolution, and phenotypic switch for escape and spread to a new host

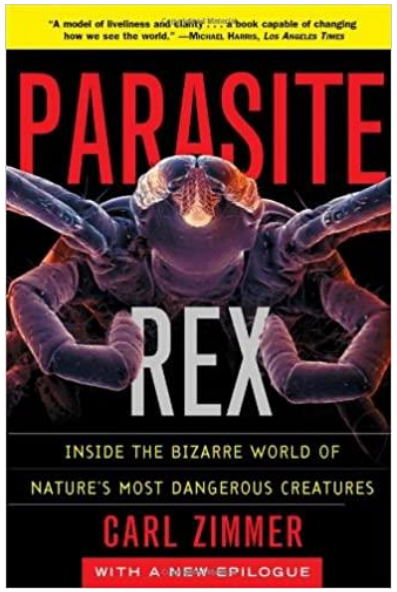
Quorum sensing

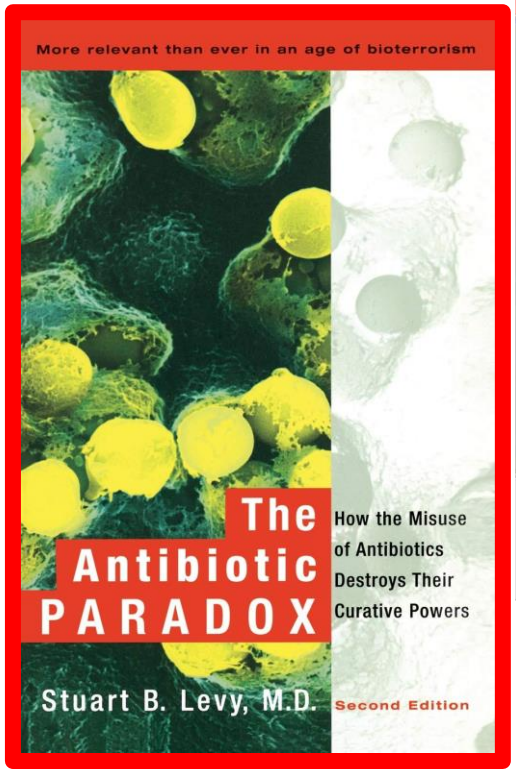


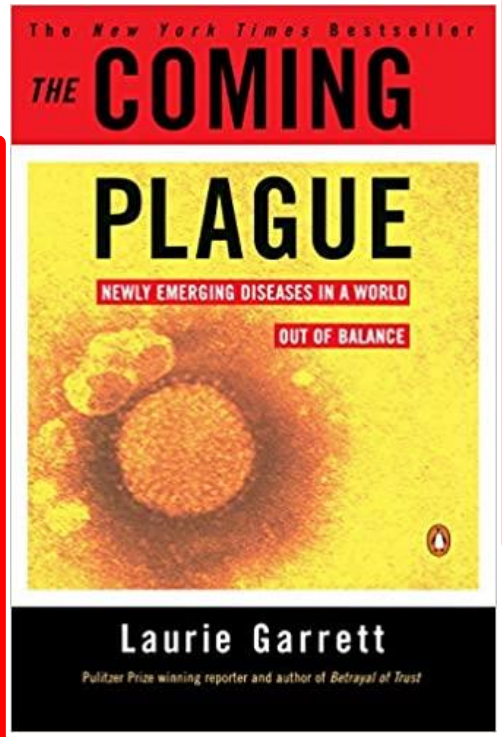
Non-textbook literature

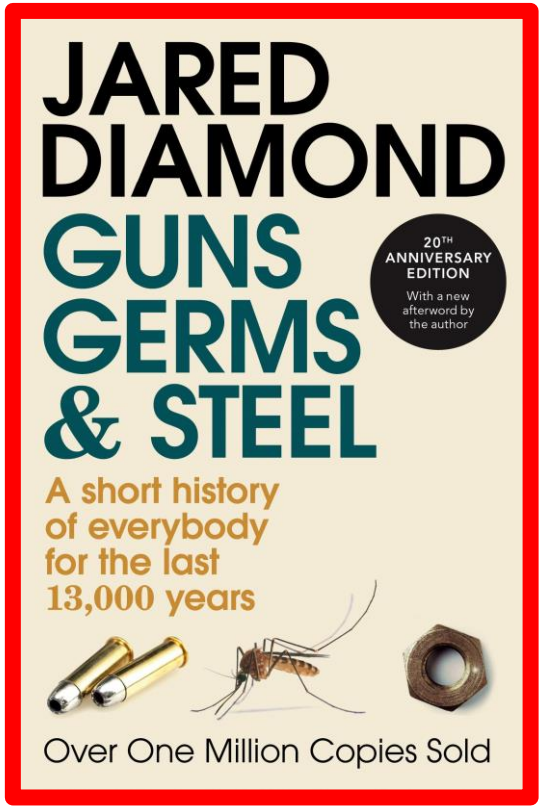












Thank you for your attention!