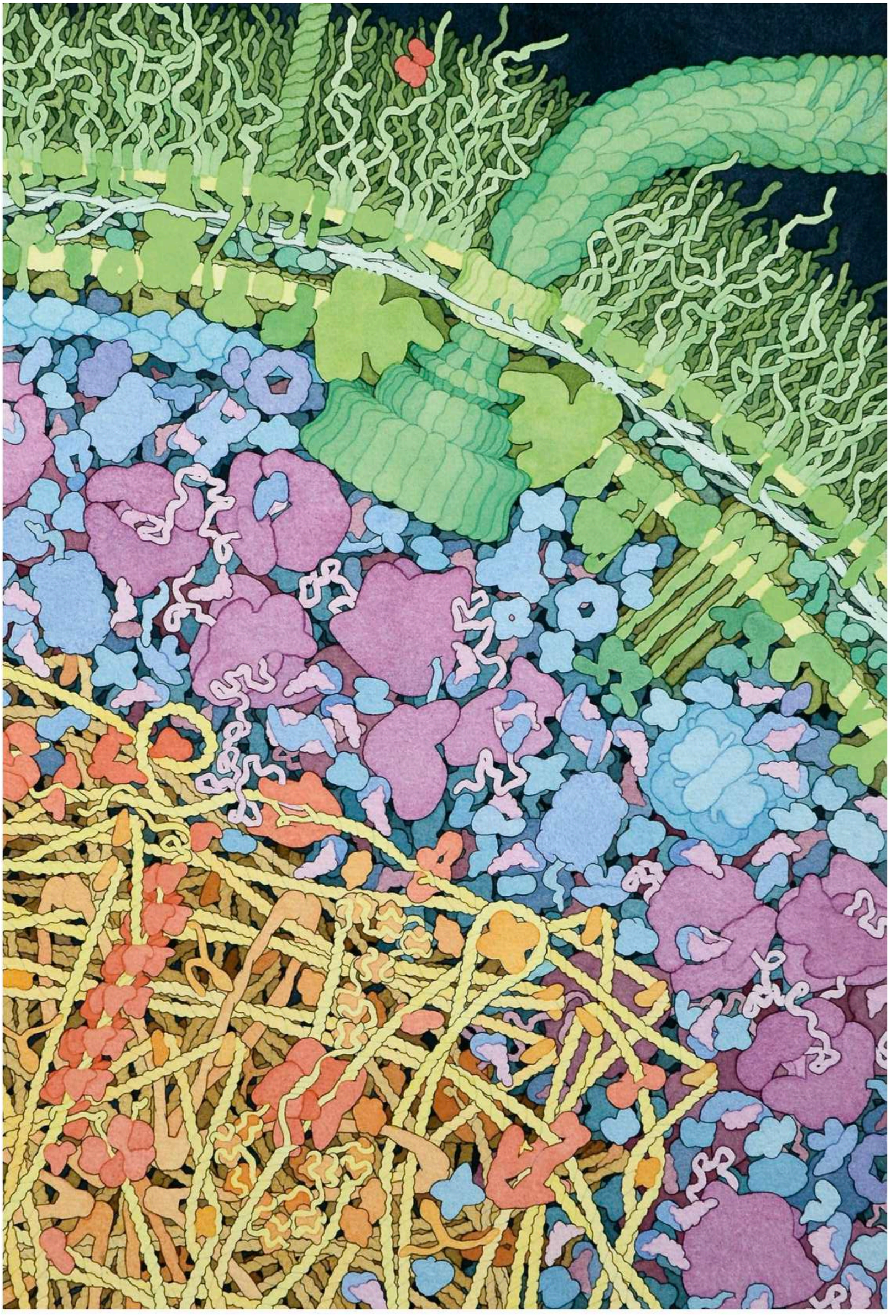
L08

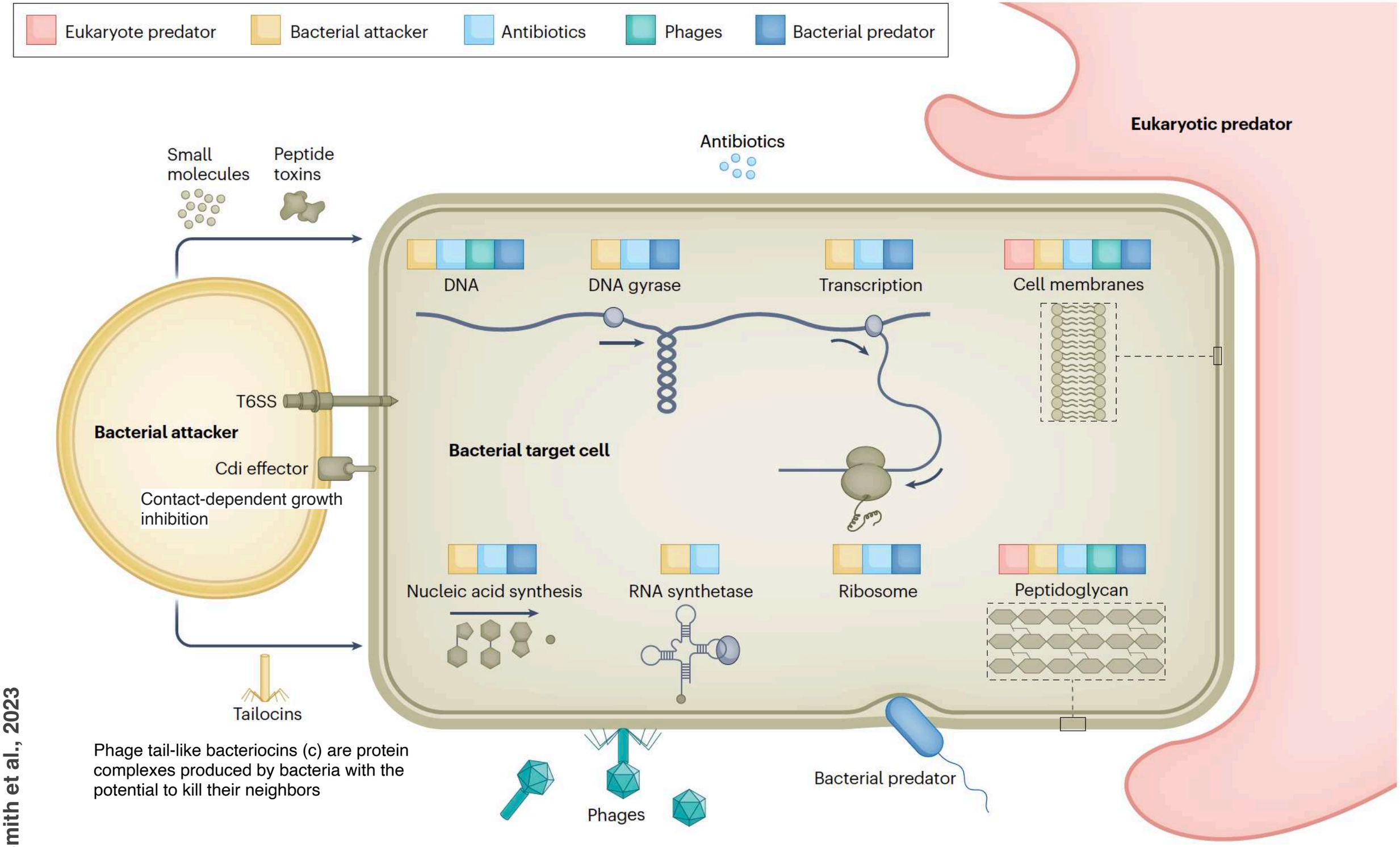
Recap

Microbial Defence

David S. Goodsell



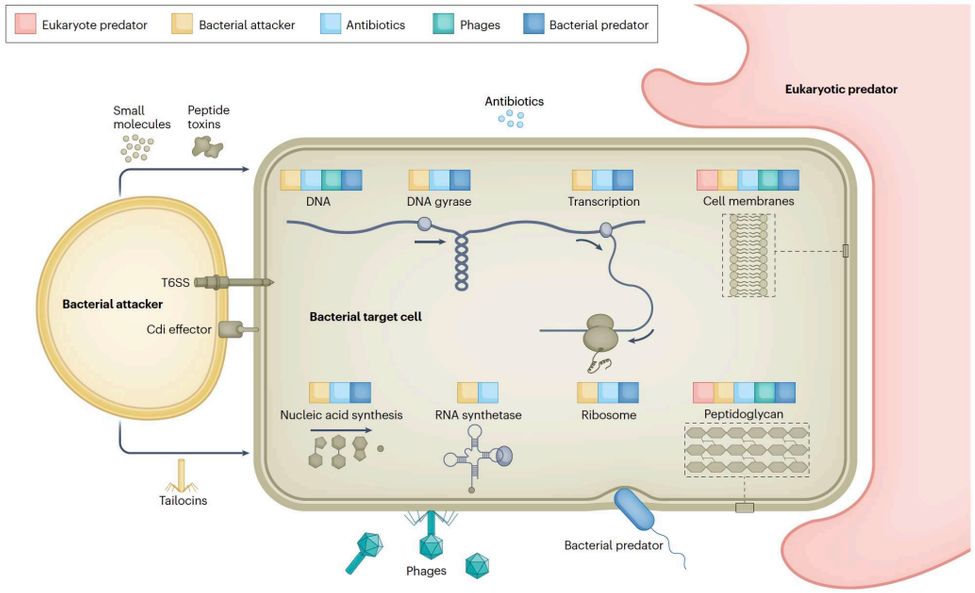
The diverse microbial threats



Phage tail-like bacteriocins (c) are protein complexes produced by bacteria with the potential to kill their neighbors

Smith et al., 2023

Smith et al., 2023



- Most attacks target **core cellular processes and functions** of the microbial cell

- Microbial competitors antagonize a target bacterium via diverse mechanisms, including both **contact-dependent** weaponry (the type VI secretion system (T6SS); **Cdi effectors**) and **diffusible weaponry** (small molecules, antibiotics, peptide toxins and tailocins)

- The majority of clinical antibiotics are also derived from bacteria and other microorganisms

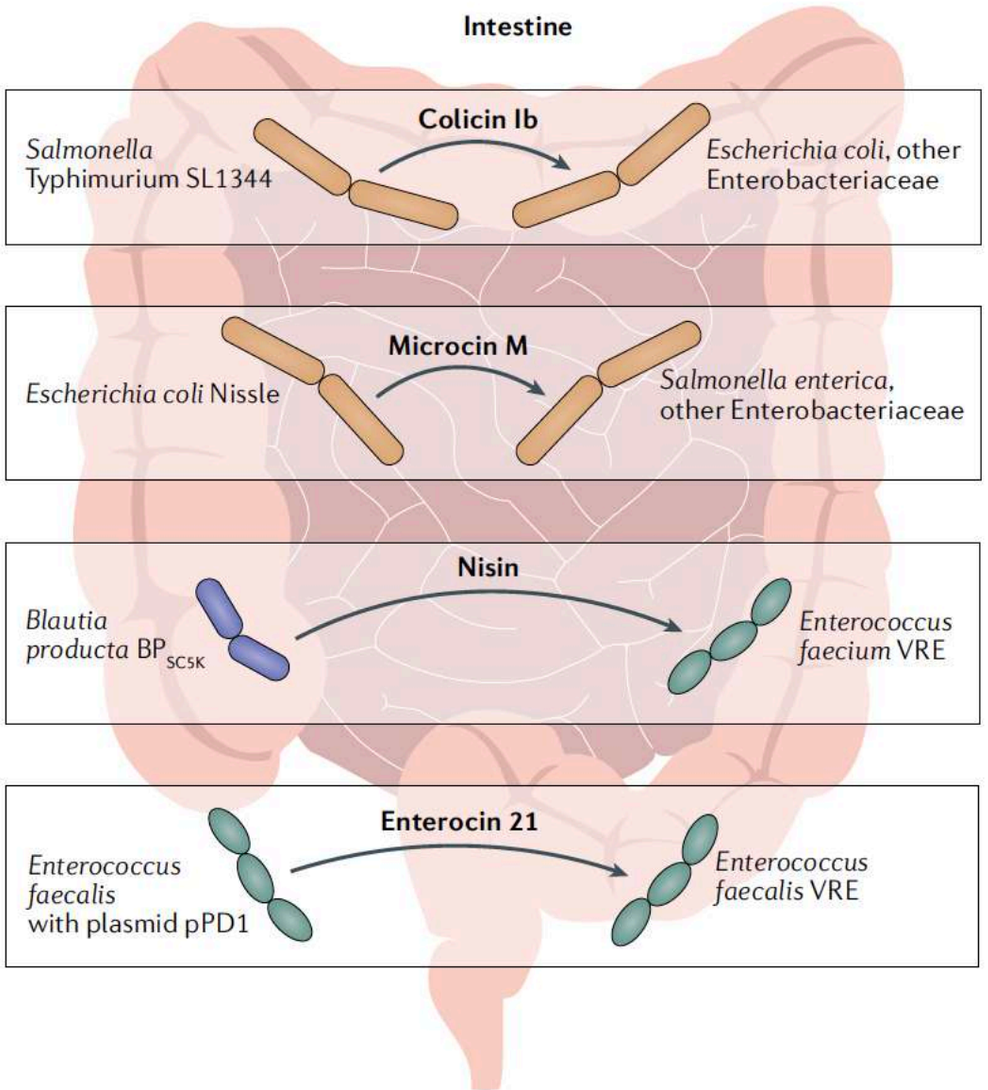
- Following infection of a bacterial cell, phages attack cell walls and membranes to release their progeny via cell lysis

- Some bacterial predators, such as *Bdellovibrio* species and similar organisms, invade the host cell periplasm, injecting toxins that digest various cytoplasmic components

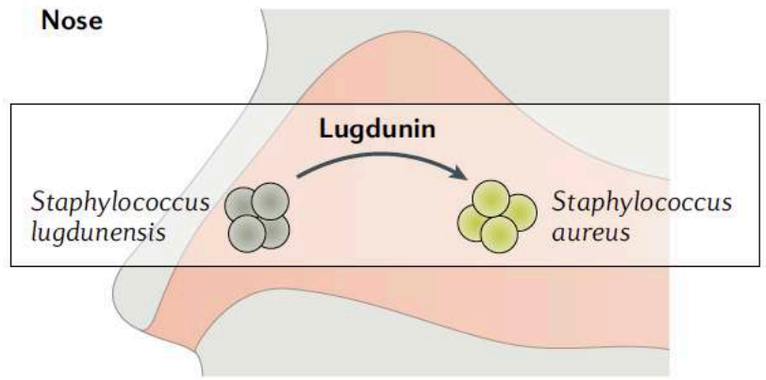
- Many eukaryotic predators **engulf and digest target bacteria whole in phagosome compartments**

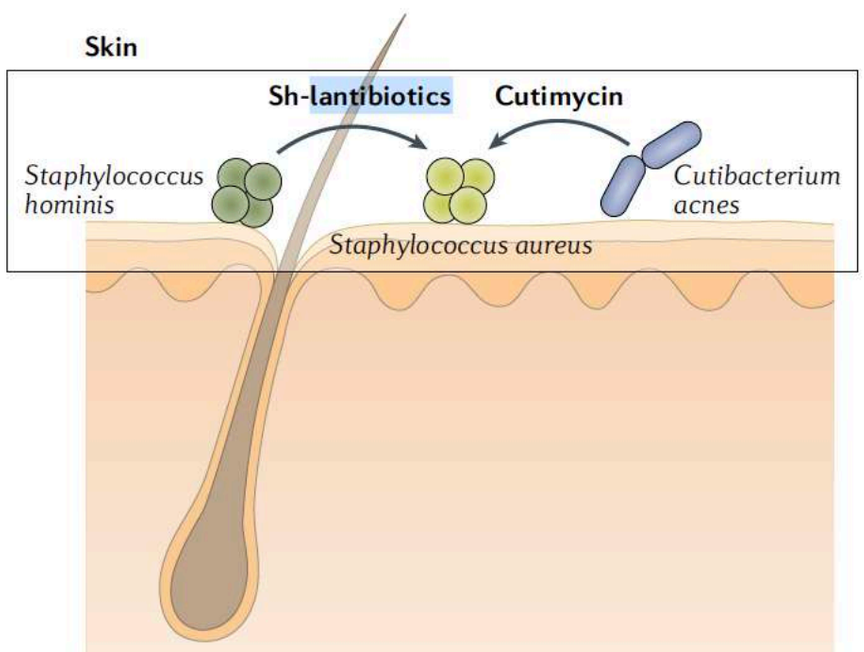
Bacteria/pathogen-targeting bacteriocins

- Many commensal bacteria produce **small antibacterial molecules** termed bacteriocins that have the capacity to eliminate specific colonizing pathogens
- **Bacteriocins have been defined as ribosomally synthesized antimicrobial proteins or peptides**, which either remain unaltered (class II bacteriocins) or are modified by enzymatic tailoring (class I bacteriocins)
- Bacteriocins target inhibition of **cell wall biosynthesis, transcription, translation, DNA replication and outer membrane biogenesis, and disruption of cell membranes**

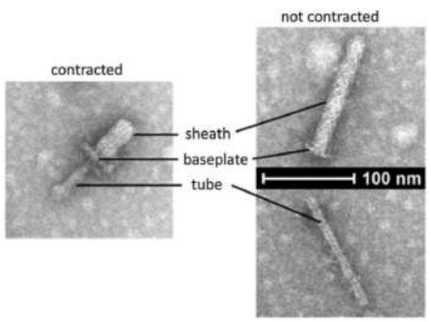


Heilbronner et al., 2021



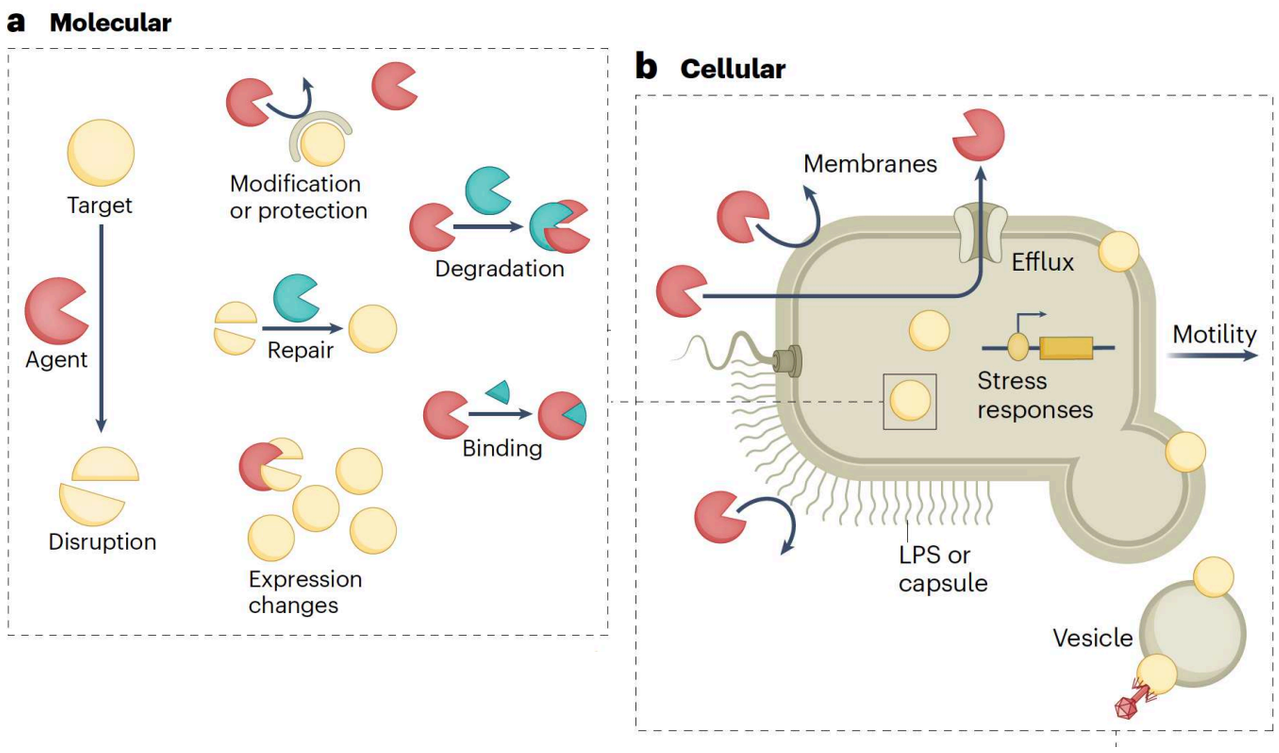


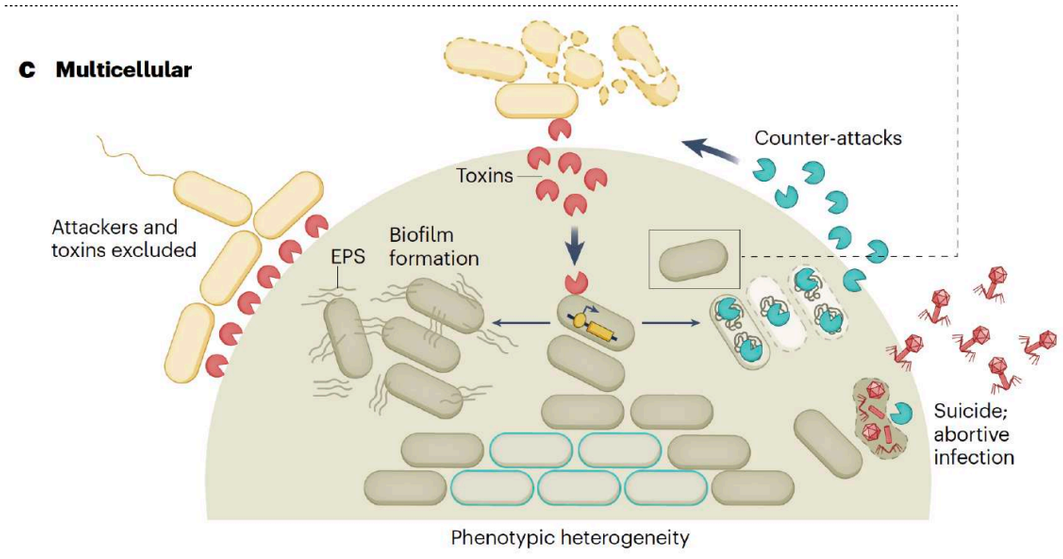
Tailocins



Patz et al., 2019

Microbial multiple lines of defence against biotic threats



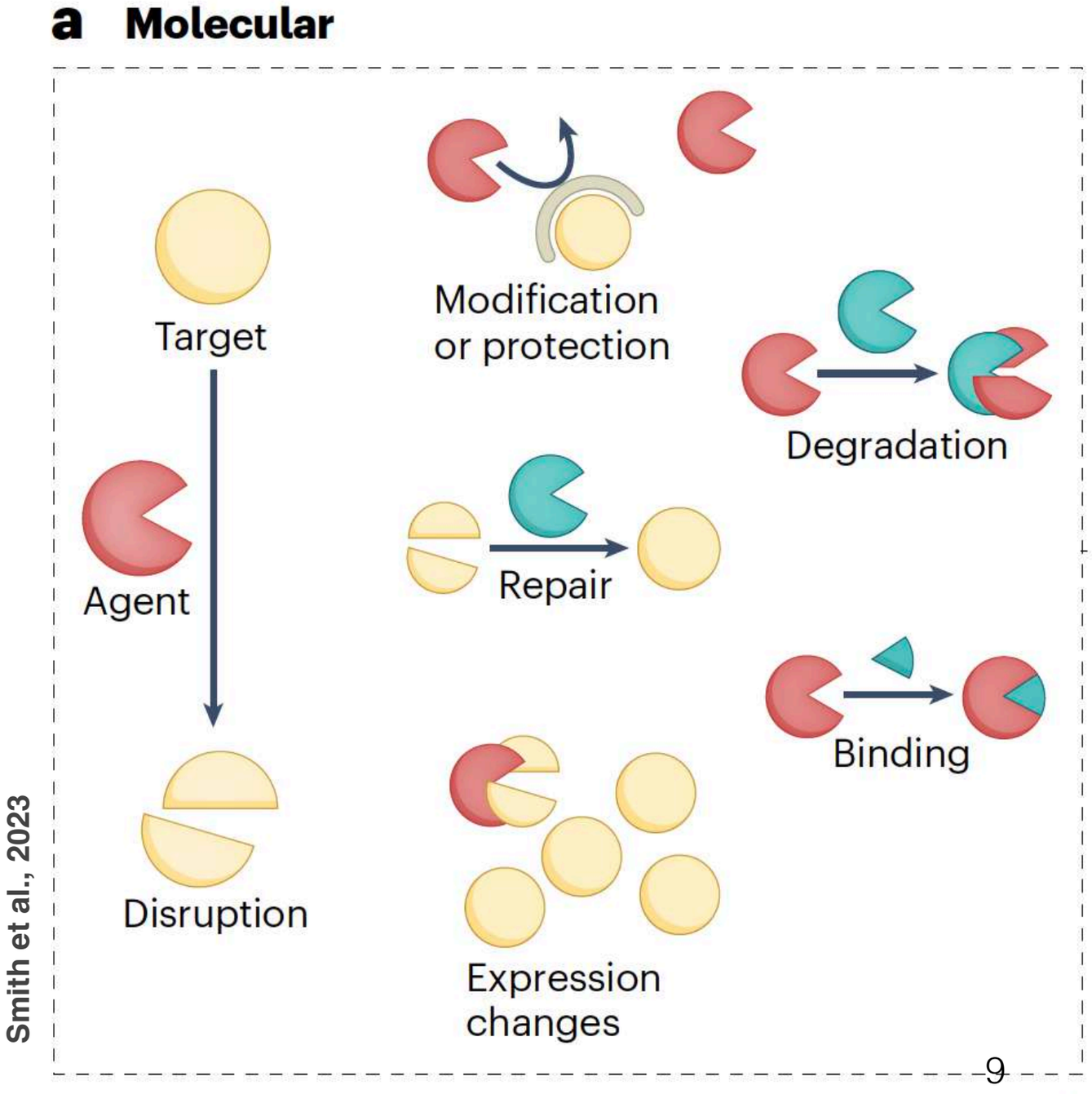


At both the **individual** and **collective** level, bacteria draw upon a plethora of defensive adaptations to escape harm

Defences operate at diverse spatial scale

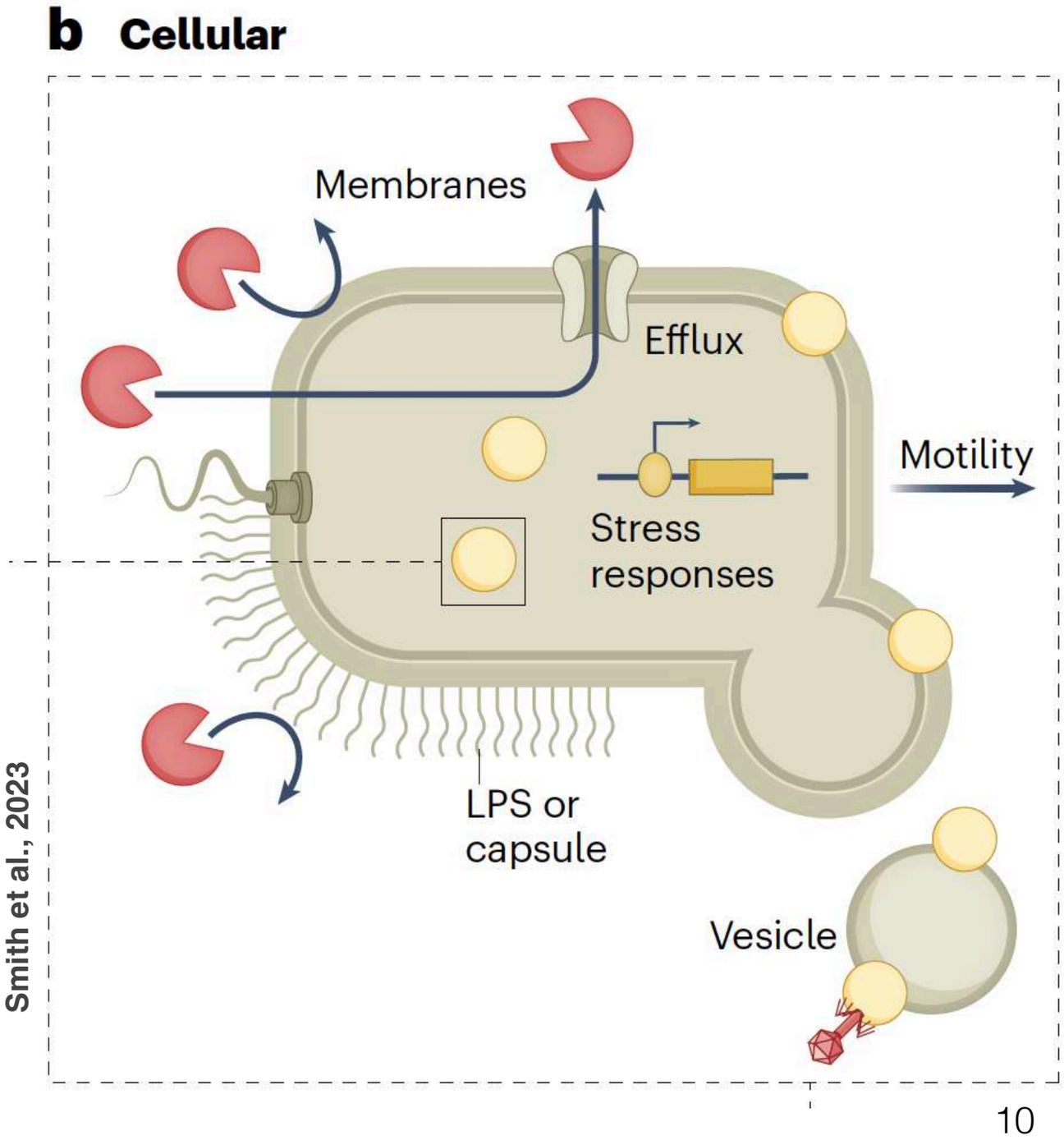
Smith et al., 2023

Microbial multiple lines of defence against biotic threats, I



- At the **molecular level**, attacks by competitors, phages and predators are mediated by harmful agents (*e.g.*,**toxins, enzymes and genetic elements**) that disrupt cellular functions by interacting with diverse targets
- Bacteria can mitigate disruption at a molecular level by **altering** the target or **compensating** for its disruption, or by **destroying or binding** to the harmful agent

Microbial multiple lines of defence against biotic threats, II

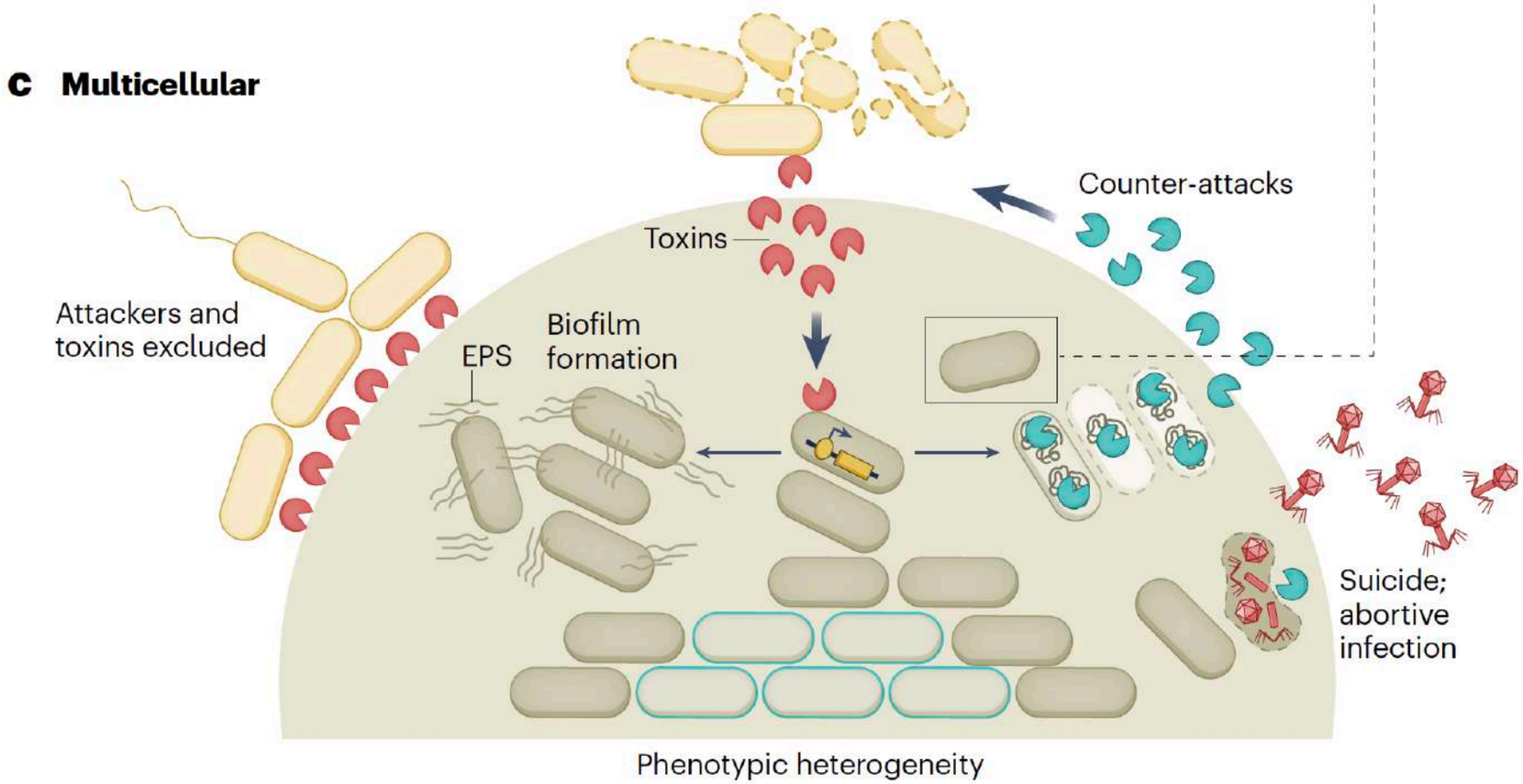


- At the **cellular level**, **macromolecular barriers**, including cell membranes, S-layers, lipopolysaccharide (LPS) or capsules, prevent harmful agents from entering a bacterial cell

- **Efflux pumps** remove harmful molecules that overcome barriers, and **motile** bacteria can escape harmful environments

- **Secreted membrane vesicles** can bind and inactivate toxins and phages

Microbial multiple lines of defence against biotic threats, III



Smith et al., 2023

- At the **multicellular level**, bacteria create collective barriers such as production of extracellular polymeric substances (EPSs) in the **biofilm** formation that exclude attackers
- Dense cell groups can limit toxin penetration via r**educed diffusion or collective degradation**
- Resistant subpopulations (**phenotypic heterogeneity)**, launch en masse counter-attacks and, in some circumstances (*e.g.*, abortive infection), commit suicide to protect kin cells
- **Stress responses** and other regulatory pathways enable these defences to be activated in response to specific or general threat cues

Eukaryotic predator

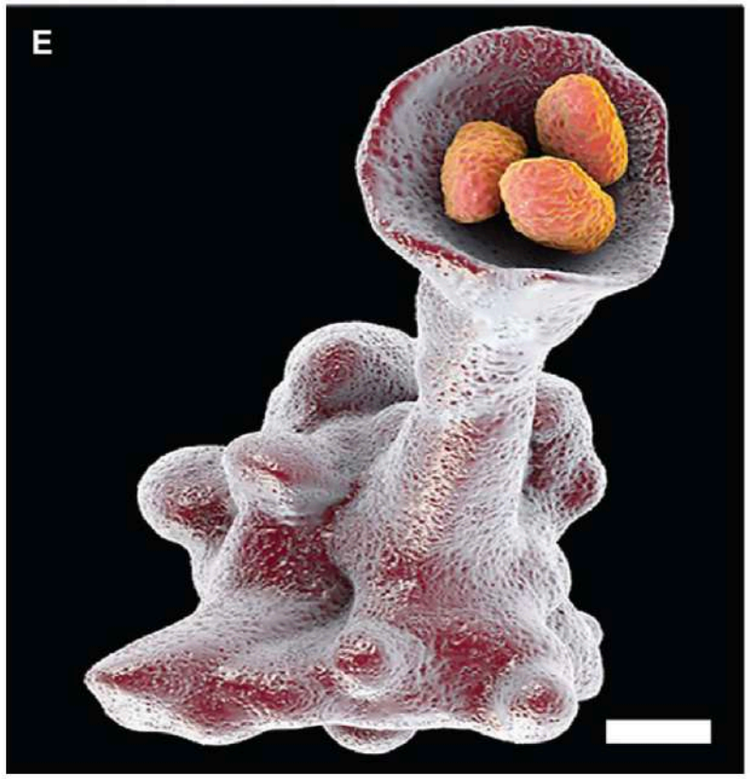
Top down control over microbial population by protists and phages

Evolutionary arm race and development of anti-predator strategies at both the **individual** and **collective** level

Anti-predator strategies such as morphological changes, increased motility, biofilm formation, production of toxic metabolites, contact dependent microscale weapon and resistance to lysosomal digestion

Phagotrophy evolved over a billion years ago, which triggered the ability of a cell to ingest a particle of organic material, whether dead or alive, as food

Myzocytosis piercing the surface of a cell and sucking out its contents as food



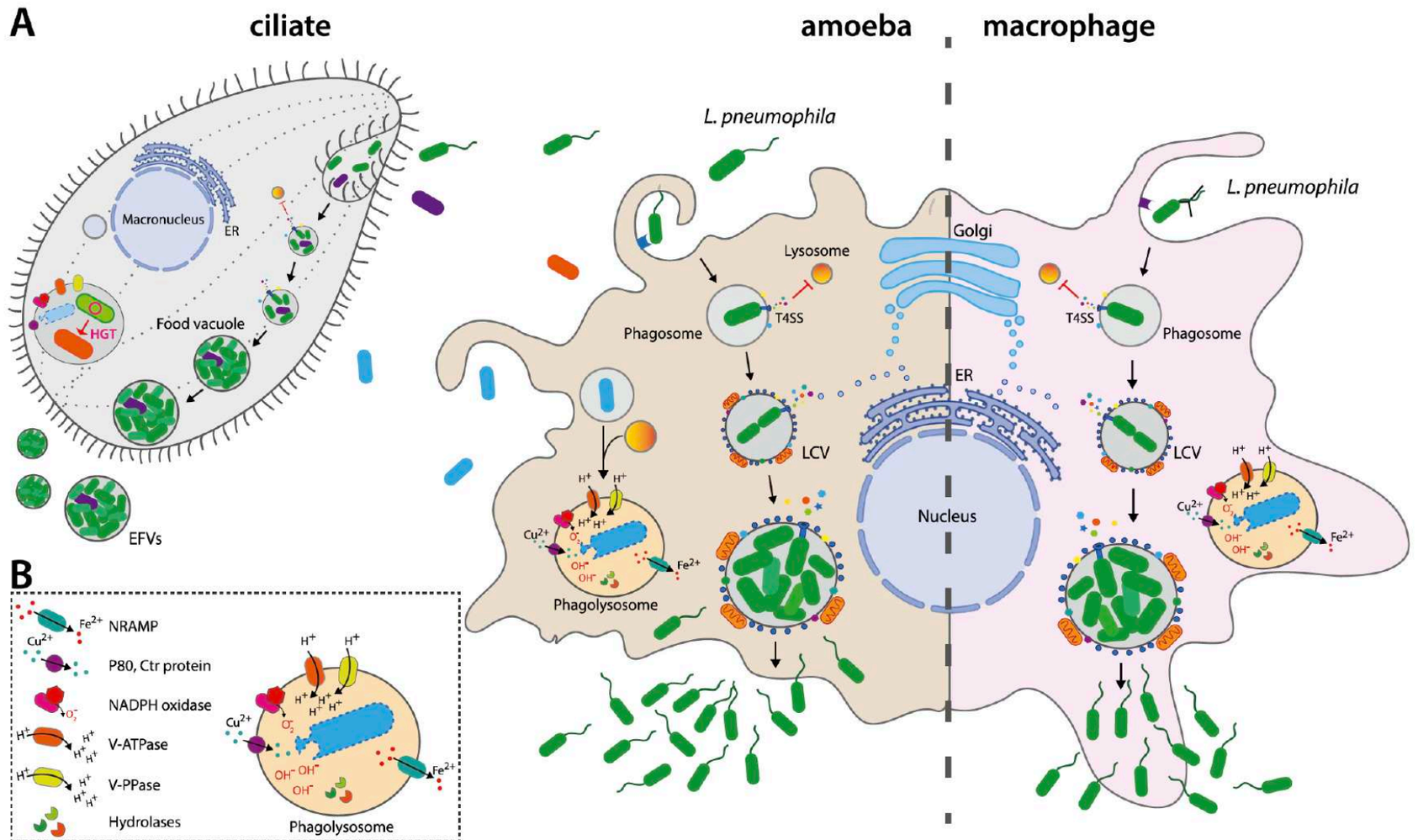
Scanning electron micrograph showing a predatory ameba using a pseudopod to envelop and consume bacteria (image purchased from Science Photo Library, scale bar 2 µm)

In the microbial world there is a convergence between anti-predator strategies and the virulence traits that possibly have evolve to increase fitness not to cause harm *per se*

Phagotrophy

Bacterium/Archaeon is trapped and engulfed in the phagosome
Fusion between phagosome and lysosome:

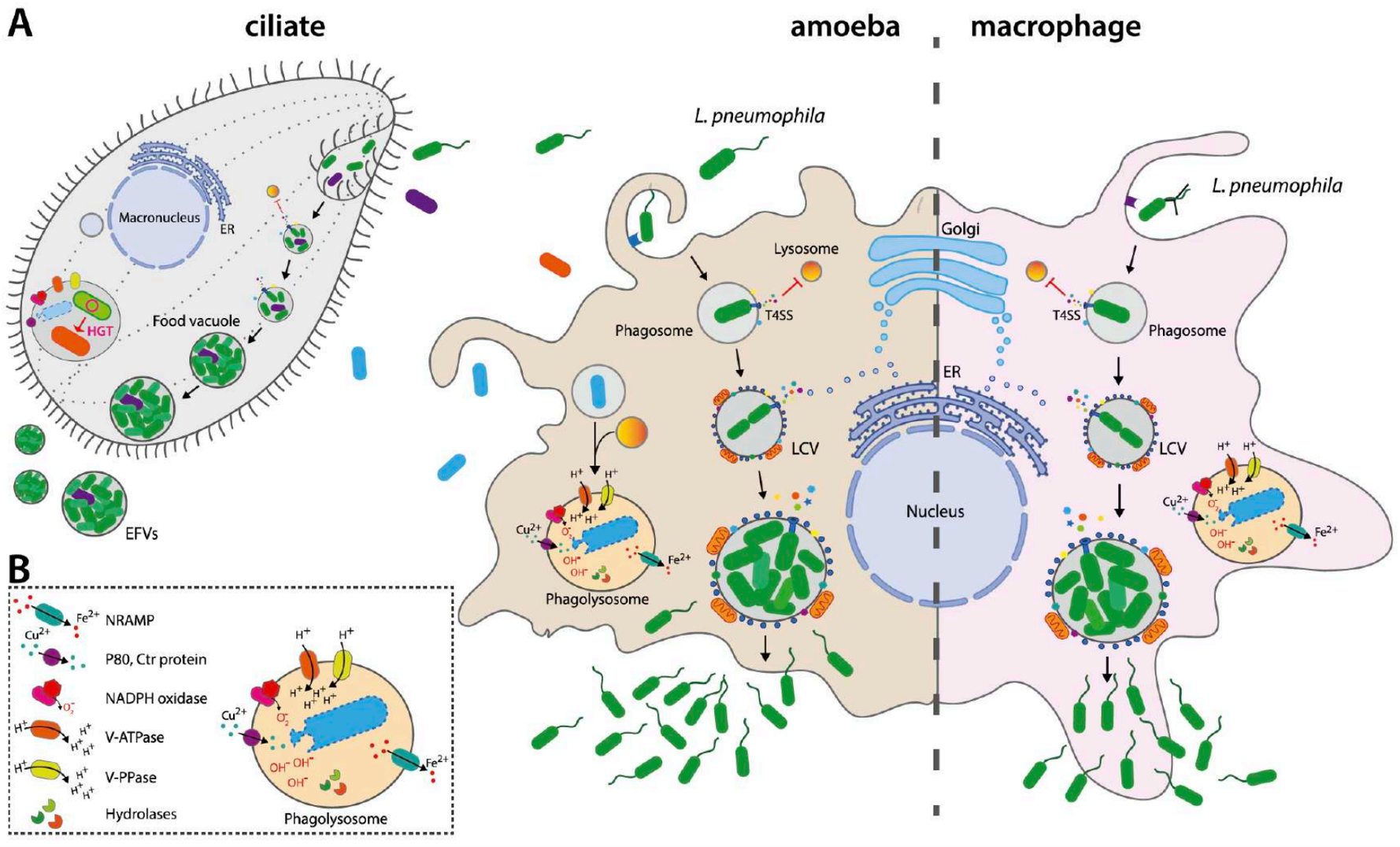
- ★ Enzymatic digestion
- ★ Phagosomal acidification
- ★ Oxidative burst
- ★ Fe^{2+} and Mn^{2+} depletion from the phagosome with efflux systems
- ★ Metal poisoning with Cu^{2+} and Zn^{2+}



Amaro and Martin-Gonzalez, 2021

Excape strategy from phagolysosome

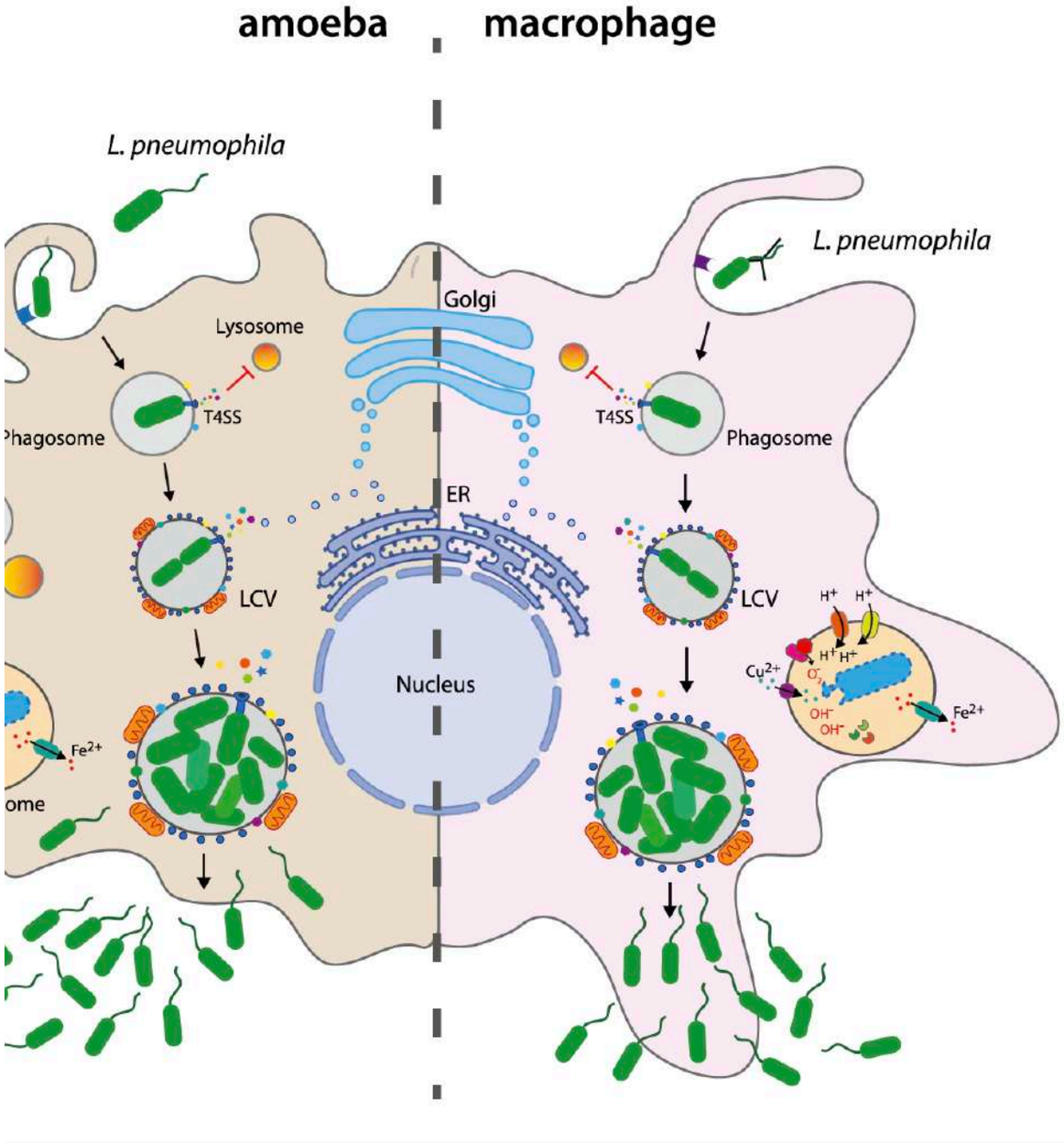
Bacteria and Archaea that **resist lysosomal digestion** in protozoa can be released into the environment freely after host cell lysis or packaged into **expelled food vacuoles (EFVs)** that serve as vectors for microbial dissemination



Amaro and Martin-Gonzalez, 2021

From a microbial point of view a macrophage is not different than a protist in the environment

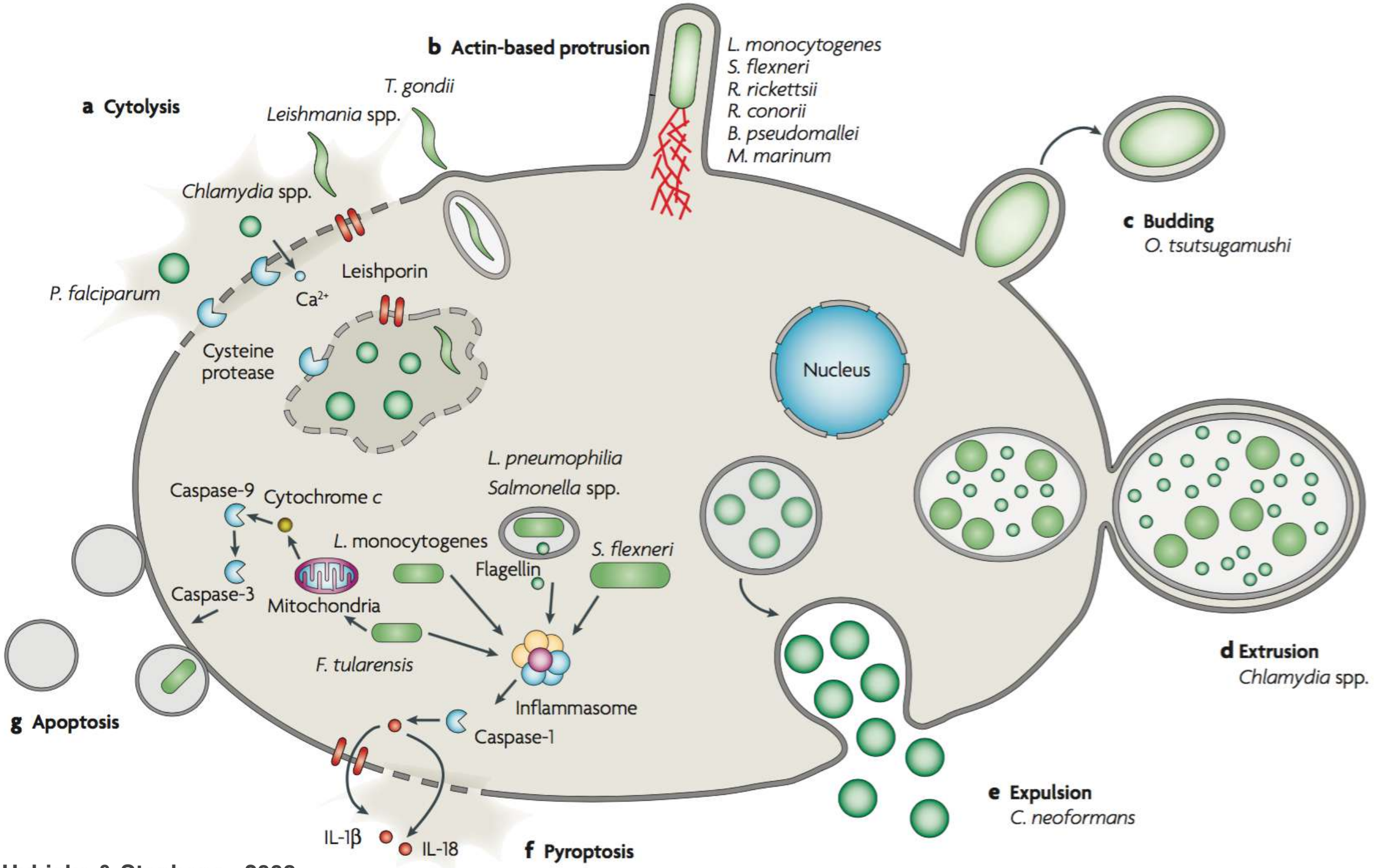
- *Legionella* is enclosed in a phagosome that neither acidifies nor fuses with the lysosome
- *Legionella* remodels it into a replicative compartment called Legionella containing vacuole (LCV)
- LCV is decorated with recruited mitochondria, RER, and ER-to-Golgi complex-derived vesicles
- After several rounds of replication, *Legionella* breaks out the LCV membrane into the cytosol before lysing the host cell



Amaro and Martin-Gonzalez, 2021

EXIT Strategies and mechanisms

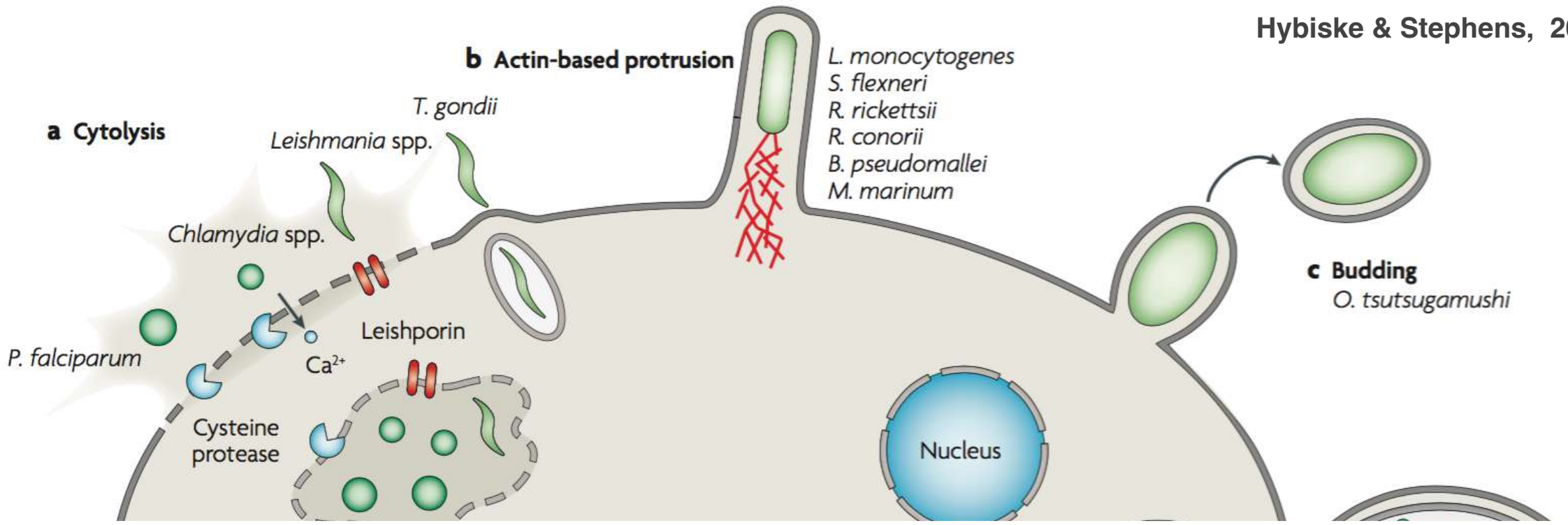
Bacterial pathogens avoid being digested in the phagolysosome and utilise the cytoskeleton and the membrane production machinery/vacuole to escape



Hybiske & Stephens, 2008

Cytolysis and Actin-based protrusion

Hybiske & Stephens, 2008



a | **The cytolysis, and destructive and sequential rupture, of the vacuole and cell membranes**. Putative mechanisms include proteases (*Plasmodium falciparum* and *Chlamydia* spp.), pore-forming proteins (PFPs) (*Leishmania* spp.) and the unique mechanism of *Toxoplasma gondii*.

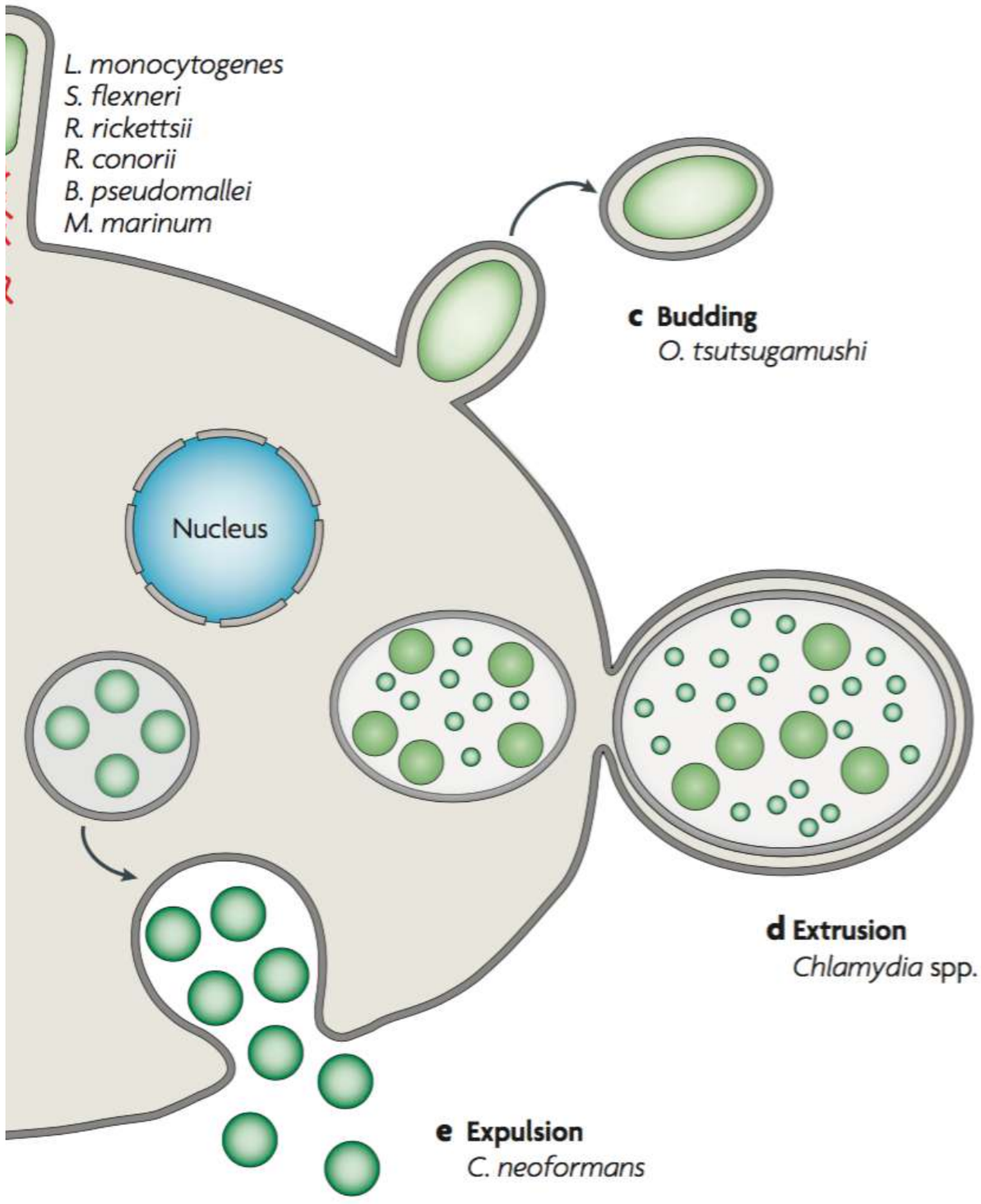
b | **Actin-based protrusion**, which is exploited by *Listeria monocytogenes, Shigella flexneri, Rickettsia rickettsii, Rickettsia conorii, Burkholderia pseudomallei* and *Mycobacterium marinum*, results in a single bacterium that **uses the force that is generated by actin polymerization to protrude** from the cell membrane and force engulfment into a neighbouring cell.

Budding, Extrusion and Phagosomal expulsion

c | The **budding** of *Orientia tsutsugamushi*, in which a single bacterium is encased by plasma membrane

d | The **extrusion** of *Chlamydia* spp., in which the large *Chlamydia*-containing **vacuole** pinches off and extrudes out of the cell; the extruded vacuole is encased by cytosol and plasma membrane

e | The **phagosomal expulsion** of *Cryptococcus neoformans*, in which the large vacuole fuses with the plasma membrane by an undefined exocytic process

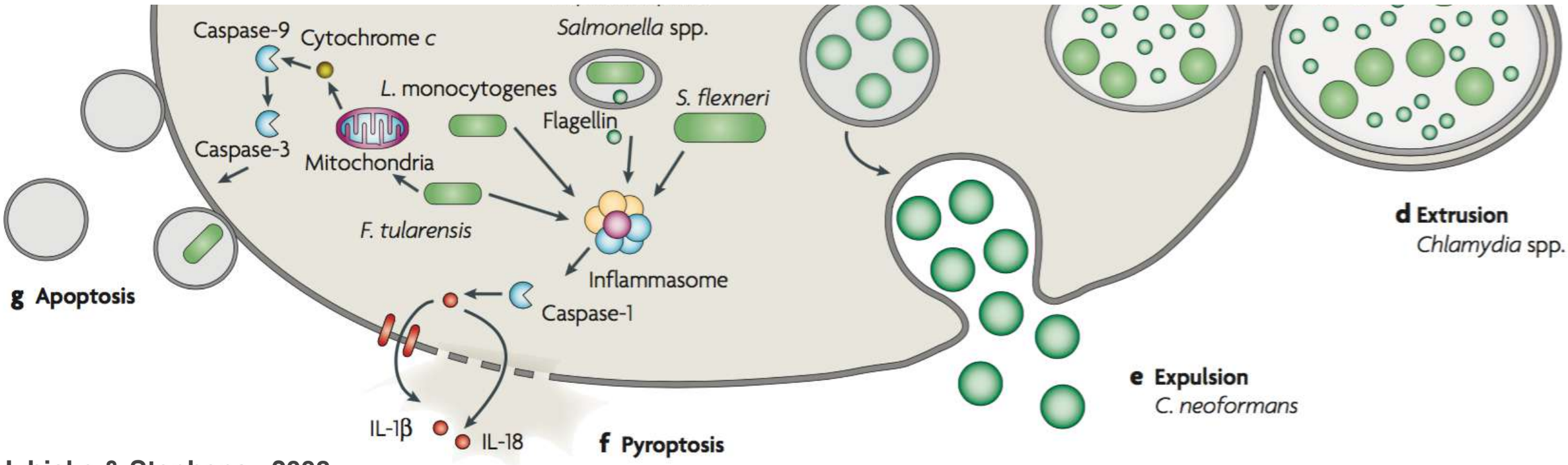


Hybiske & Stephens, 2008

Pyroptosis and Apoptosis

f | **Proinflammatory pyroptosis** is defined by the sensing of bacterial molecules (flagellin of *Legionella pneumophila* and *Salmonella* spp. and unknown molecules of *S. flexneri*, *L. monocytogenes* and *Francisella tularensis*) through the host **inflammasome**. The inflammasome proteolytically activates caspase-1, which leads to interleukin (IL)-1β and IL-18 activation and secretion. Cytokine secretion occurs initially through a caspase-1-dependent pore, and is then released upon necrotic cell lysis.

g | **Apoptosis** is induced by *F. tularensis* using the intrinsic pathway of activation — cytochrome *c* release from mitochondria and activation of the initiator caspase-9 and the effector caspase-3. The bacterial molecule (or molecules) that is responsible for apoptotic induction is unknown.



Hybiske & Stephens, 2008

Competition for metals: siderophores

- Siderophores are secondary microbial metabolites that are synthesized through biosynthetic pathways involving several enzymes, including non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs)

- **Siderophores scavenge Fe and other metals**: Cu, Co and Ni, toxic metals such as Al, Ga and Pb or radionuclides such as U

- Fe and other metals are important for the **metabolism** such as ETC and enzyme structure and functionality

EVERY ORGANISMS NEED Fe AND (certain) METALS (*e.g.*, microbes, Euk, macrorganisms and humans)

- Once synthesized, siderophores are **excreted** into the **niche environment** to **scavenge** iron and other metals to transport it back into the bacteria through **specialized transporters**

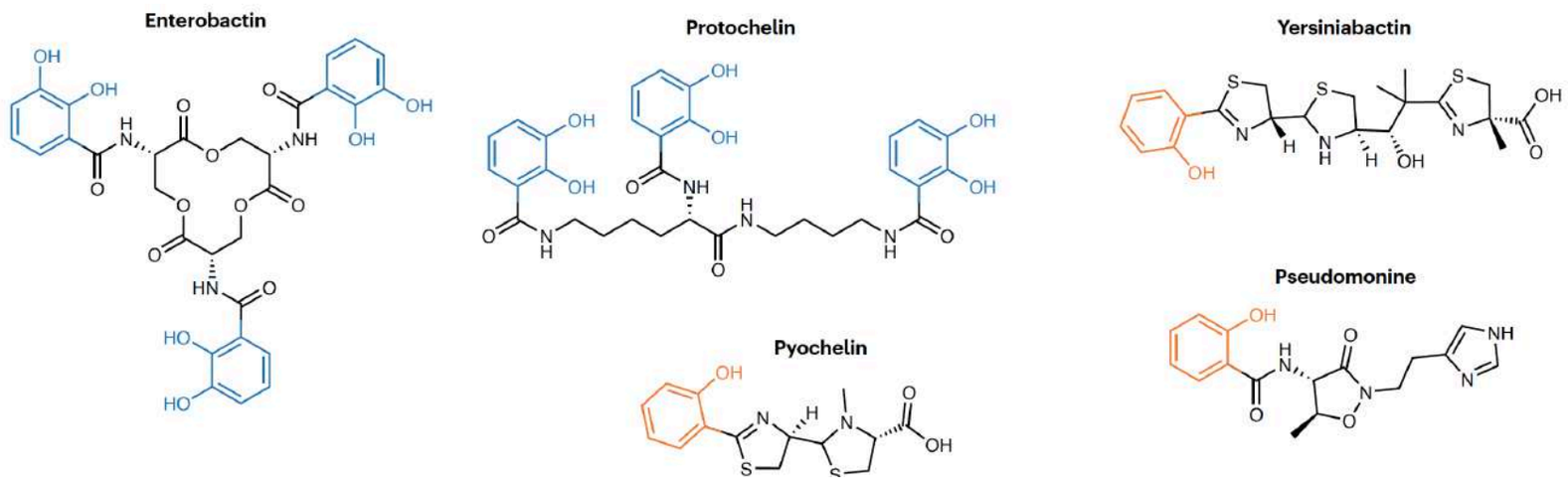
- Metal/iron release from siderophores in the bacterial cells occurs via various molecular mechanisms

- Microorganisms can produce two to three different siderophores, and they possess the ability to exploit siderophores that have been produced by other microorganisms **(xenosiderophores)**

Competition for metals: siderophores

- Siderophores have diverse affinity for metals
- Molecular weights ranging from 200 Da to 2,000 Da
- For example:
    - The formation constants for the hydroxamate siderophore desferrioxamine B with Ga^{3+}, Al^{3+} and In^{3+} range between 10^{20} M^{-1} and 10^{28} M^{-1}, whereas that with Fe^{3+} is 10^{30} M^{-1}
    - The formation constants for pyoverdine with Zn^{2+}, Cu^{2+} and Mn^{2+} fall between 10^{17} M^{-1} and 10^{22} M^{-1}, whereas that with Fe^{3+} is 10^{32} M^{-1}
-

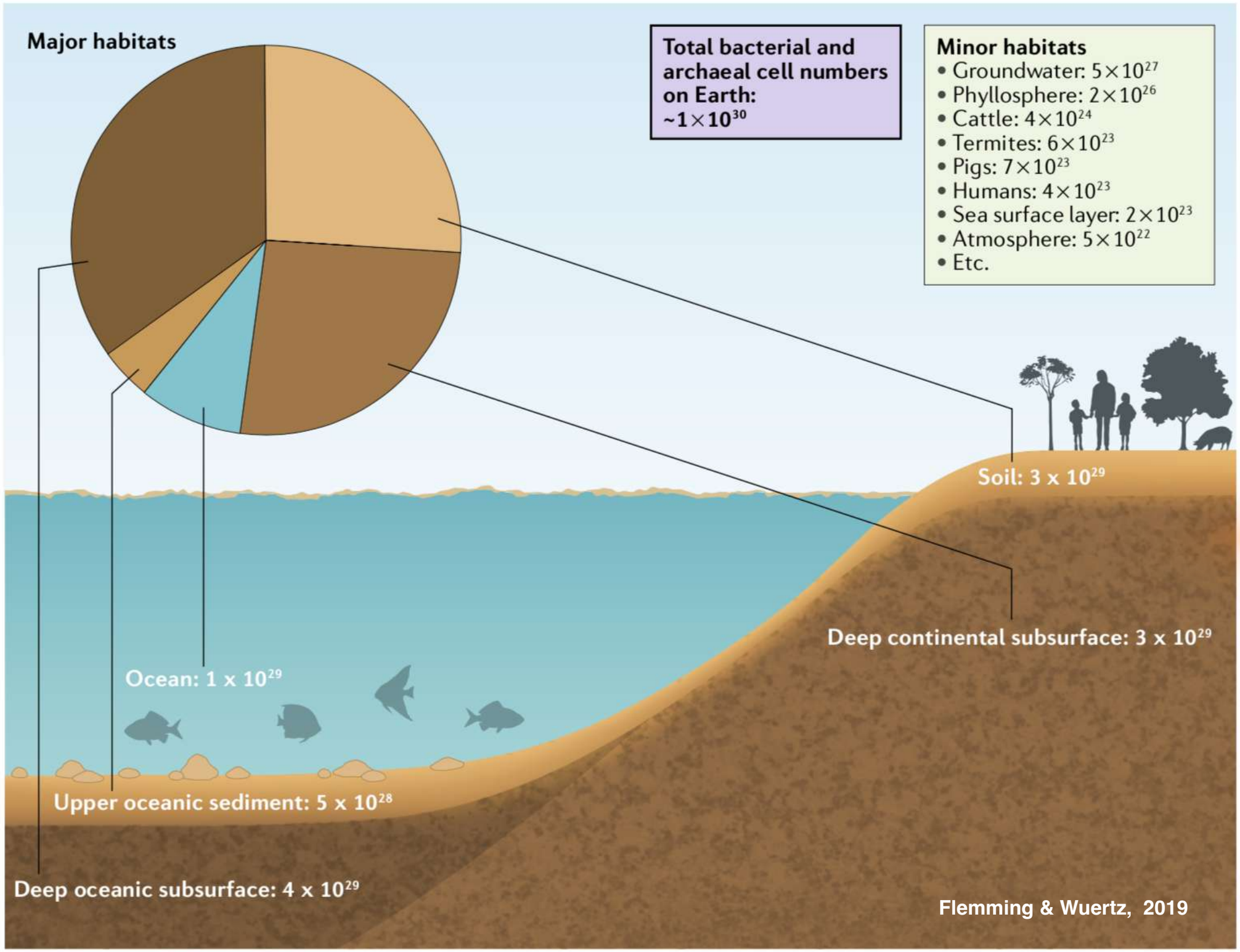
Schalk et al., 2024



Microbes-Humans interactions:

Microbial pathogenesis & OneHealth

Earth is inhabited by 10^{11}–10^{12} microbial species (Locey & Lennon, 2016)



~1,400 known species of human pathogens (bacteria, virus, fungi, protozoa)

Microbial pathogenesis and virulence

- Bacterial **pathogenesis** is the process by which bacteria infect (mechanism of infection) and cause disease in a host (mechanisms of disease development)
- **Not all bacteria are pathogens**
- **Ability for pathogenesis is also known as virulence**
- **Virulence** describes the organism's propensity to cause disease, through properties such as invasiveness and toxin production
- Only a small percentage of the world's bacteria cause infection and disease
- Bacterial infections have a **large impact** on public health
- Human pathogens account for much **less than 1%** of the total number of microbial species on the planet

Microbial infection and disease

- A **pathogen** is a micro-organism that has the potential to cause disease
- An **infection is the invasion and multiplication** of pathogenic microbes in close association with host's tissues within an individual or population
- Infection is distinguished from **disease**, a morbid process that does not necessarily involve infection
- **Disease** is when the infection causes **damage** to the individual's vital functions or systems
- *An infection does not always result in disease!*
- To cause an **infection, microbes must enter human bodies**
- The site at which they enter is known as the **portal of entry**

Portal of entry, I

Microbes can **enter** the human/animal body through:

A. **Respiratory** tract (mouth and nose) *e.g.*, influenza virus which causes the flu

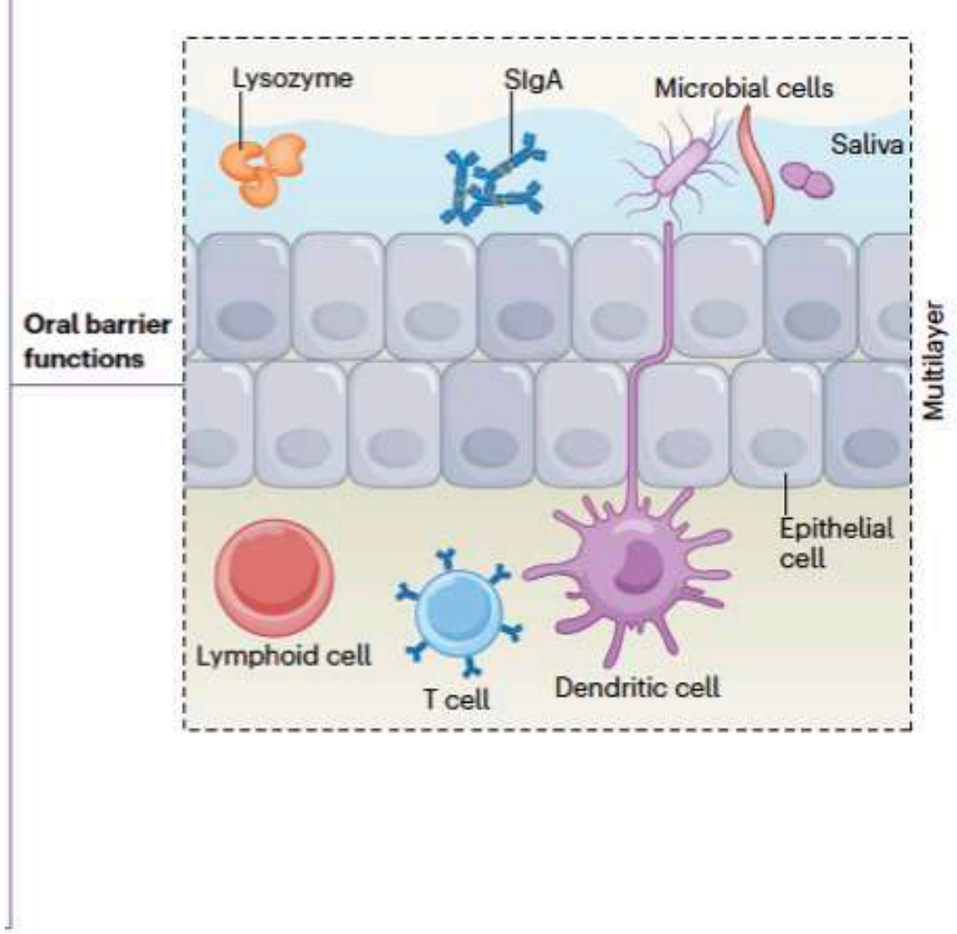
B. **Gastrointestinal** tract (mouth oral cavity) *e.g.*, *Vibrio cholerae* which causes cholera

C. **Urogenital** tract *e.g.*, *Escherichia coli* which causes cystitis

D. **Breaks in the skin surface (into blood)** *e.g.*, *Clostridium tetani* which causes tetanus

Portal of entry architecture

- Physical and chemical barriers
- Multiple layer of serrated dead cells
- Dividing cells
- Cilia (movement) and mucus production
- pH change
- Enzymes, immunoglobuline, AIs, bacteriocins, and siderophores
- “Our tamed symbiotic protists”: the immune system cell armies



Kunath et al., 2024

Portal of entry, II

Bacteria: Human Microbiome, Infection & Spread – Microbiology | Lecturio

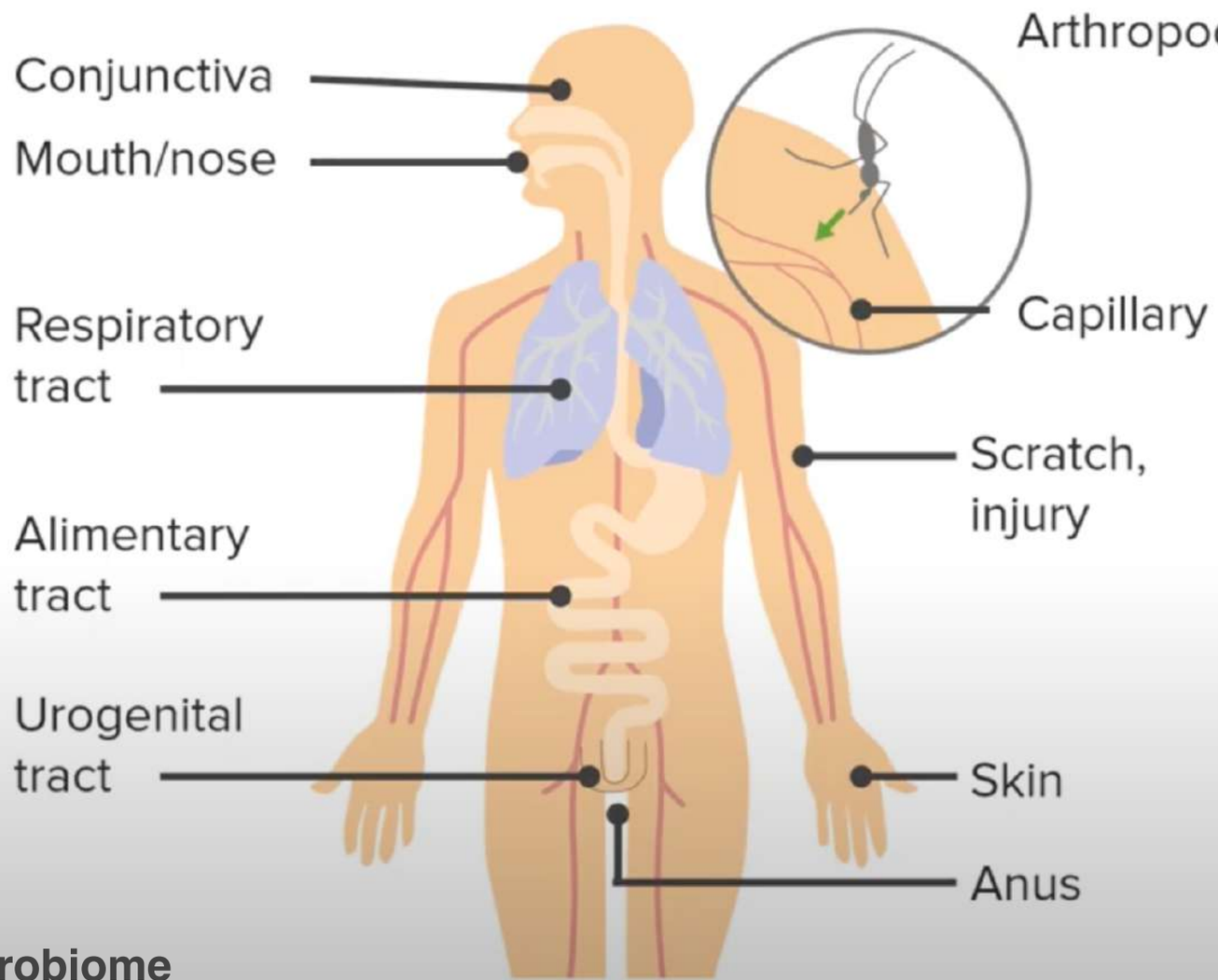
Gaining Entry

Mucous membranes

- Breathing, eating, sex
- Cholera, whooping cough, gonorrhea

Penetration

- Invasion into cells, tissues
- Insect bites
- Scratch, injury

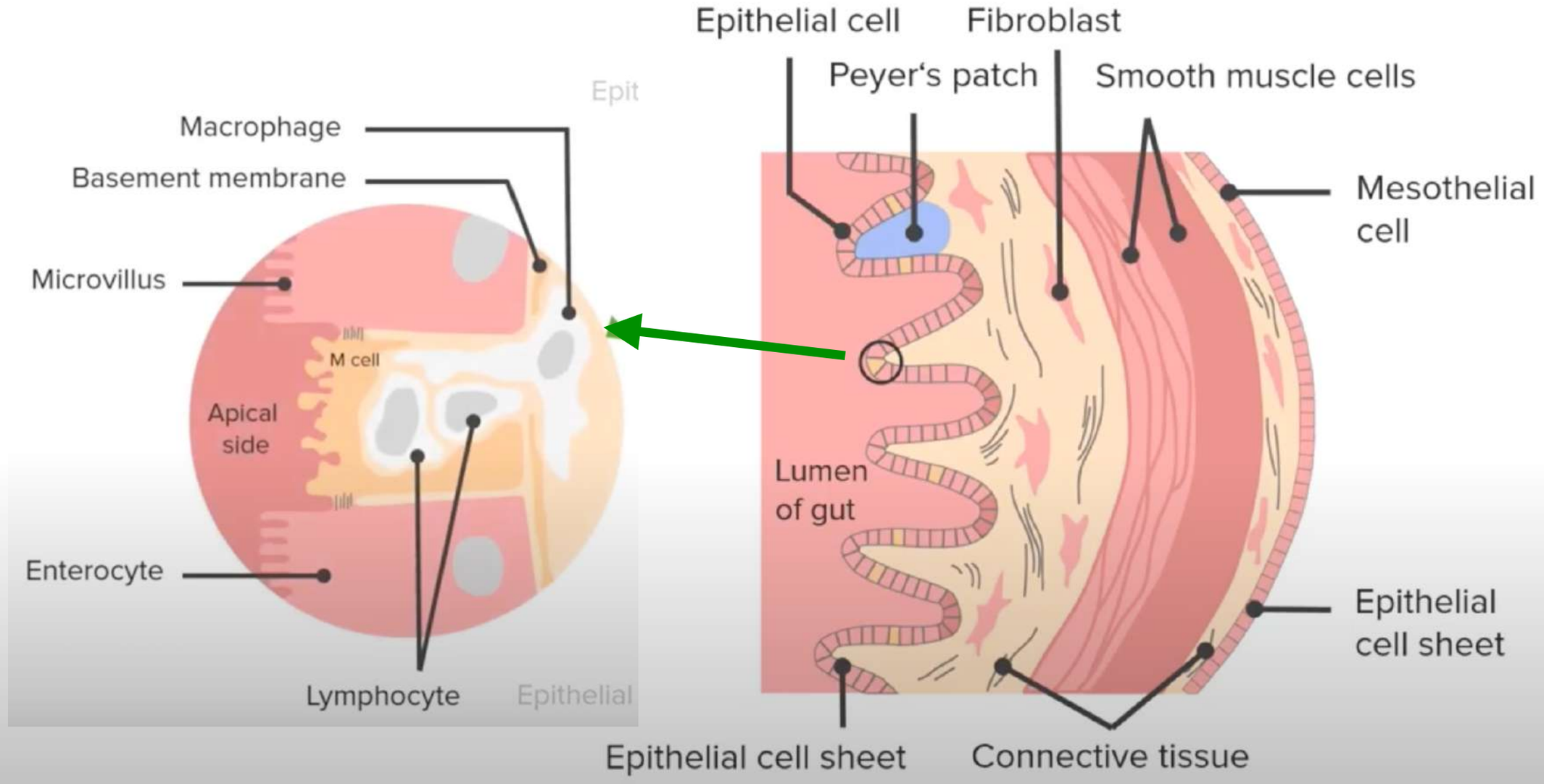


Prof. Dr. Vincent Racaniello; http://lectur.io/microbiome

Portal of entry, III
Gastrointestinal tract

Bacteria: Human Microbiome, Infection & Spread – Microbiology | Lecturio

Spread



Prof. Dr. Vincent Racaniello; http://lectur.io/microbiome

Microbial infection dynamics

To make HUMANS, ANIMAL, PLANTS ill/sick microbes have to:

A. Reach their **target** site in the body

B. **Attach** to the target site they are trying to infect so that they are not dislodged

C. Multiply rapidly

D. **Obtain** their **nutrients** from the host

E. Avoid and survive attack by the host's immune system

ROUTES OF TRANSMISSION

- The **spreading** of microbes is called **transmission**

Transmission involves the following stages:

A. Escape from the host or reservoir of infection (where the infectious agent normally lives and multiplies)

B. Transport to the new host

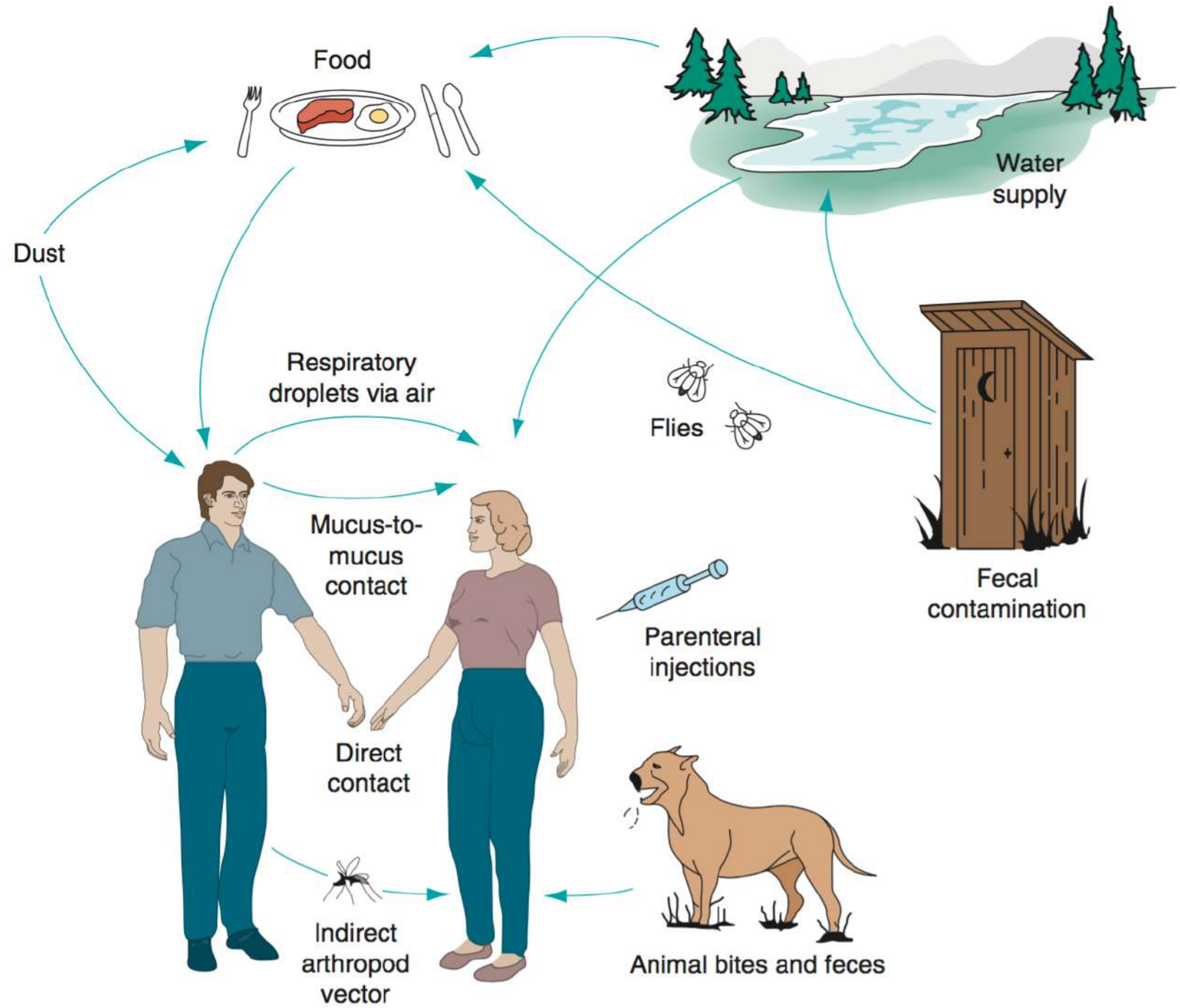
C. Entry to the new host

D. Escape from the new host

E. Different pathogens have different modes of transmission —> For example respiratory pathogens are usually airborne and intestinal pathogens are usually spread by water or food

Mode of disease transmission

Understanding mechanisms of transmission is important not only because it helps control those diseases that emerge but also because it provides opportunities to control multiple diseases transmitted by the same mechanisms



Doreen & Gorbach, 2008

Figure 5 Modes of disease transmission. Reproduced with permission from Engelkirk PG and Burton GR (eds.) (2006) Epidemiology and public health. In: *Burton's Microbiology for the Health Sciences*, 8th edn., ch. 11. Baltimore: Lippincott Williams and Wilkins.

Source of water contamination

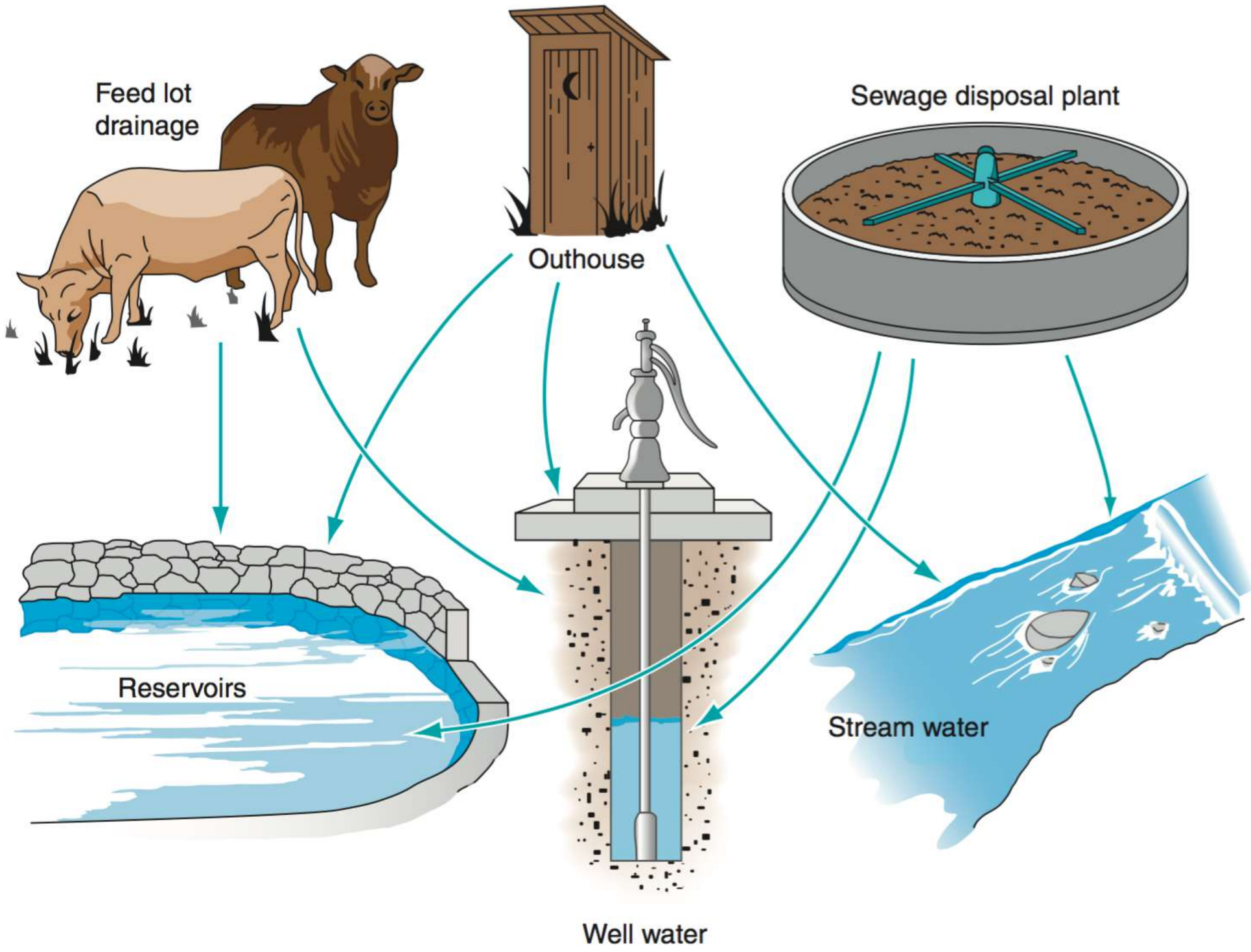


Figure 4 Sources of water contamination. Reproduced with permission from Engelkirk PG and Burton GR (eds.) (2006) Epidemiology and public health. In: *Burton's Microbiology for the Health Sciences*, 8th edn., ch. 11. Baltimore: Lippincott Williams and Wilkins.

Table 1 Reservoirs for bacteria

Reservoirs	*Disease examples*
Human	Typhoid fever, syphilis
Animal	Anthrax (cows), *Salmonella* (turtles), tularemia (rabbits), Lyme disease (white-footed mice)
Arthropods	Rocky Mountain spotted fever (ticks), endemic typhus (fleas), scrub typhus (mites)
Air	Tuberculosis
Soil	Tetanus, botulism, gas gangrene
Food	Vibrio, *E. coli* 0157:H7
Water	Shigella, Legionella

Doreen & Gorbach, 2008

Table 2 Modes of transmission of bacterial infections

Mode of transmission	*Disease examples*
Contact	Streptococcal impetigo (skin-to-skin), gonorrhea (mucus membrane-to-mucus membrane), *Salmonella* (fecal–oral), syphilis (transfusion)
Airborne	Tuberculosis, Q fever, legionella
Droplet	Pertussis, meningococcus, *Haemophilus influenzae*
Vectors	Lyme disease (tick), Shigella (fly) epidemic typhus (lice), bubonic plague (fleas)
Vehicular	*Campylobacter* (food), trachoma (fomites)

Doreen & Gorbach, 2008

MICROBIAL BATTLEFIELD

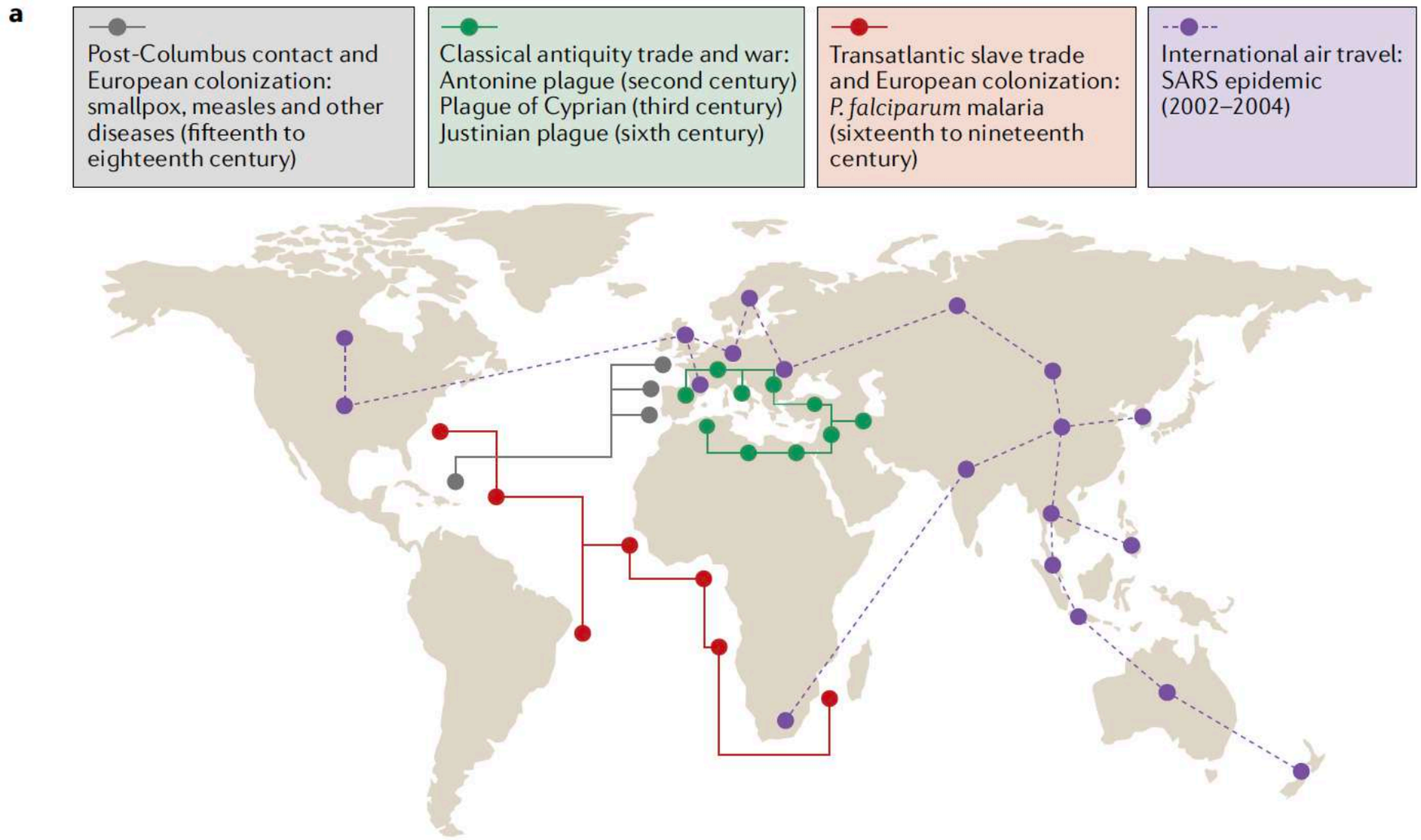
- An infection can be seen as a battle between the invading pathogens and the host
- Human bodies are equipped to fight off invading microbes that may cause disease
- **The immune response has to be tightly controlled to ensure a clearance of the microbes but also to prevent tissue damage and necrosis as result of sepsis**

- **Human natural defences are:**

1. **Aspecific defense: chemical and physical barriers**
2. **Costitutive / innate**
3. **Adaptive / inducible**

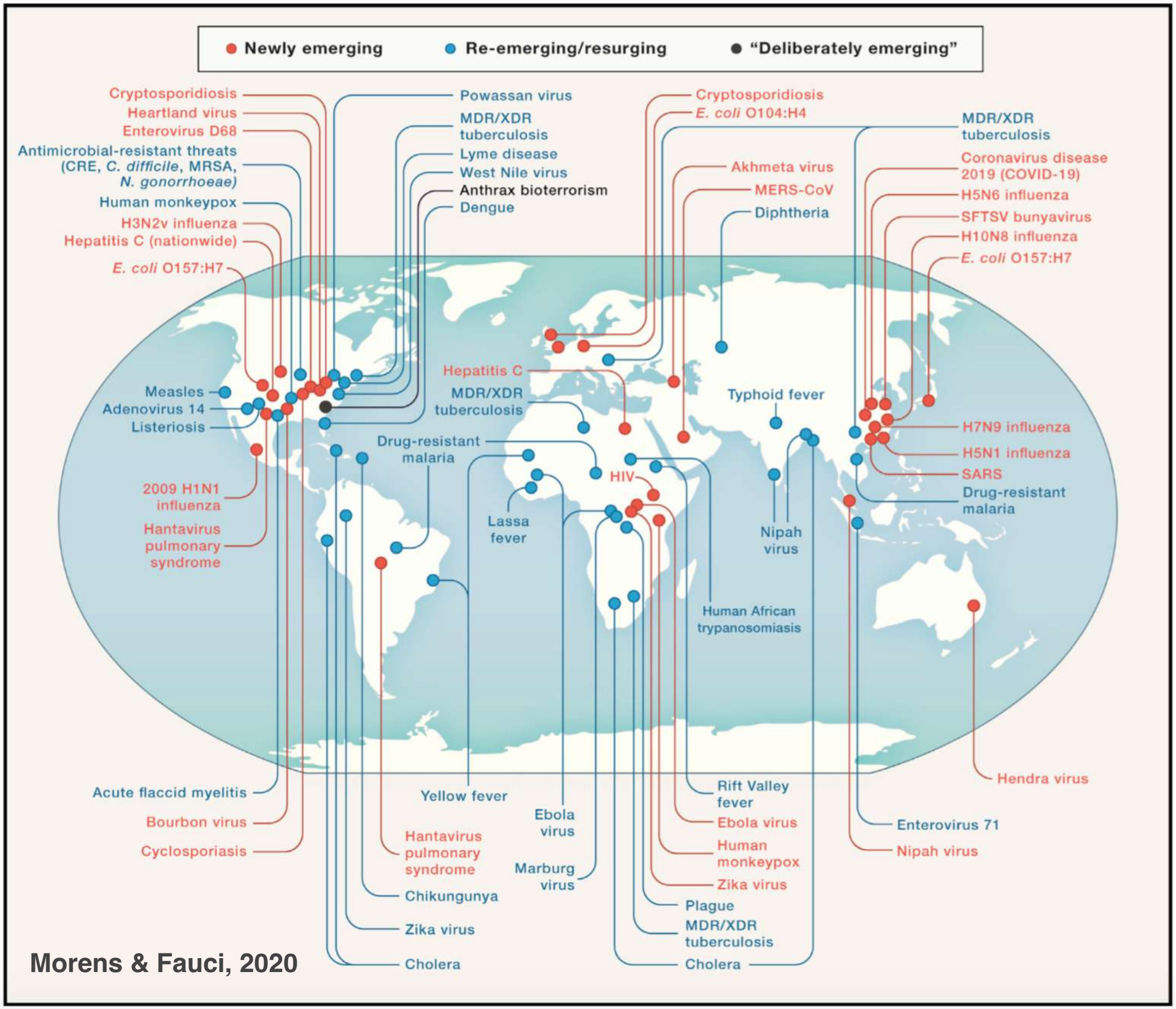
One Health

Human connectivity and infectious disease outbreaks in premodern and modern times



Backer et al., 2022

Infectious disease from 1981 to 2020



Morens & Fauci, 2020

One Health -UN Sustainable Development Goals

One Health High-Level Expert Panel (OHHLEP) defines that One Health

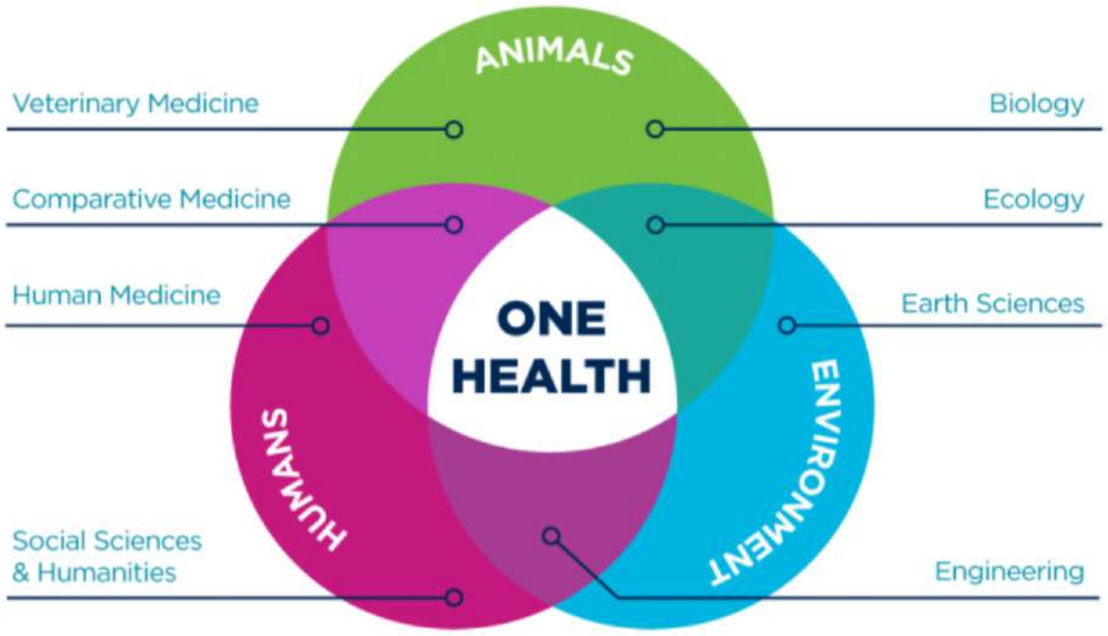
an **integrated**, **unifying** approach that aims to **sustainably** balance and optimize the **health of people, animals and ecosystems**

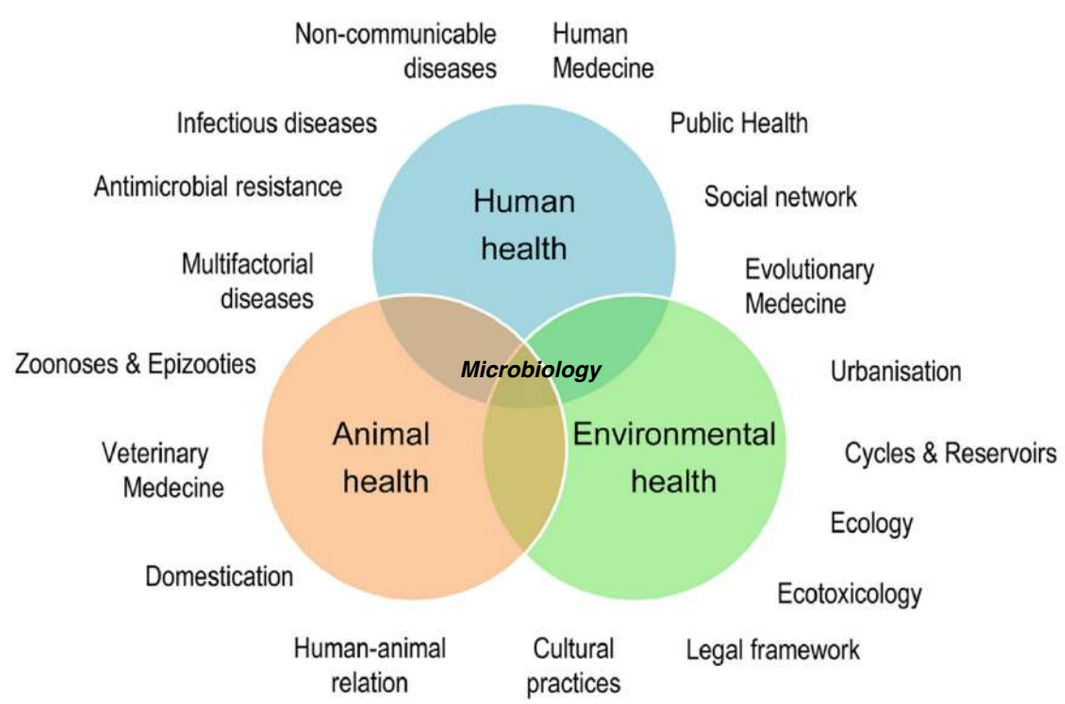
One Health recognizes that the **health** of **humans**, domestic and wild animals, plants and the wider environment (including ecosystems) is closely linked and **interdependent**

The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and **tackle threats to health and ecosystems**, while addressing the collective need for **healthy food, water, energy and air, taking action on climate change** and contributing to sustainable development

One Health: approach to designing and implementing **programs, policies, legislation and research** in which multiple sectors communicate and work together **to achieve better public health outcomes**

Holistic approach where interactions matter





Destoumieux-Garzon et al., 2018

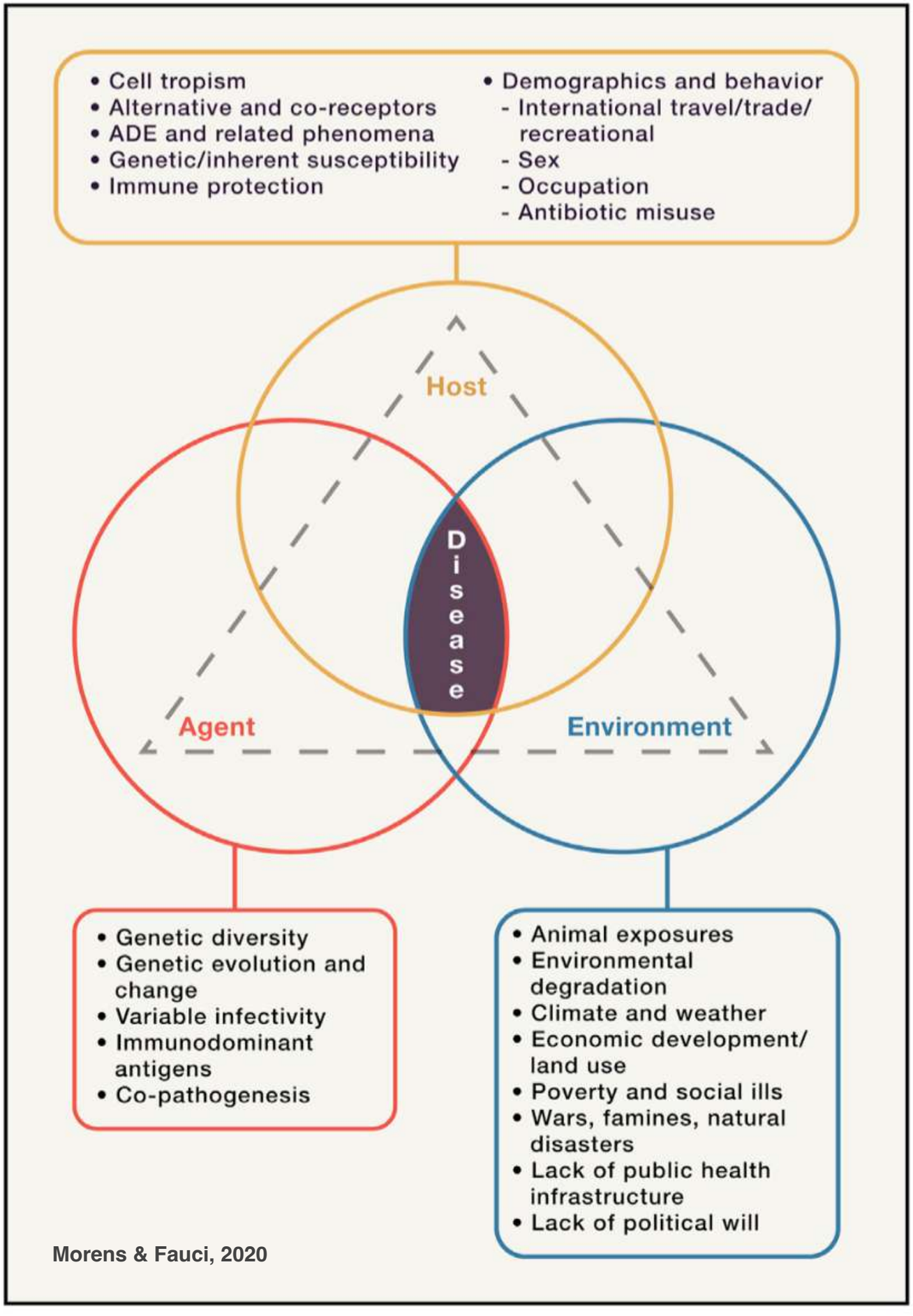
Original author not found

One Health issues include **zoonotic diseases, antimicrobial resistance**, food safety and food security, vector-borne diseases, environmental contamination, and other health threats **shared** by people, animals, and the environment

https://youtu.be/qm8NnL582uc?si=k_HGm_-TdzxDZAEl

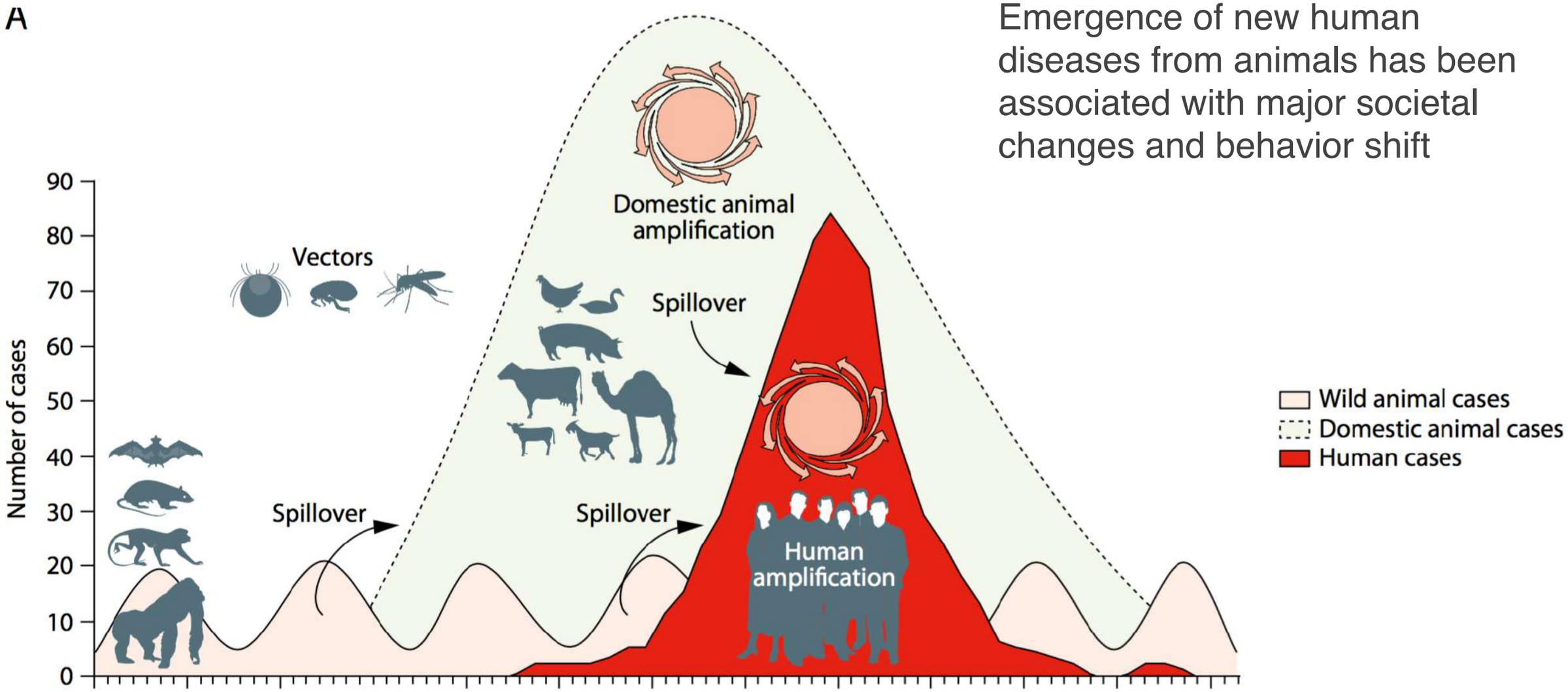
Disease landscape within interaction among host, agent and environment

- Diseases, including emerging diseases, **result** from **interactions** between infectious agents, hosts, and the environment
- Several factors lead to the development of bacterial infection and disease



Morens & Fauci, 2020

Zoonoses dynamic



Emergence of new human diseases from animals has been associated with major societal changes and behavior shift

Transmission of infection and amplification in people (bright red) occurs after a pathogen from wild animals (pink) moves into livestock to cause an outbreak (light green) that amplifies the capacity for pathogen transmission to people

United Nations Environment Programme and International Livestock Research Institute (2020)

Definition of emerging infectious diseases

Table 2. Major Categories of Emerging Infectious Diseases

Newly emerging infectious diseases	Diseases recognized in humans for the first time, e.g., HIV/AIDS (1981), Nipah virus (1999), SARS (2002), MERS (2012), COVID-19 (2019)
Re-emerging infectious diseases	Diseases that have historically infected humans but continue to re-appear either in new locations (e.g., West Nile in the United States and Russia in 1999) or in resistant forms (e.g., methicillin-resistant *Staphylococcus aureus*)
Deliberately emerging infectious diseases	Diseases associated with intent to harm, including mass bioterrorism
Accidentally emerging infectious diseases	Diseases created by humans that are released unintentionally, e.g., epizootic vaccinia and transmissible vaccine-derived polioviruses

Not included are currently established endemic diseases that are presumed to have been newly emerging at some time in the past and then went on to develop long-term persistence in human or animal populations (see text).

One Health

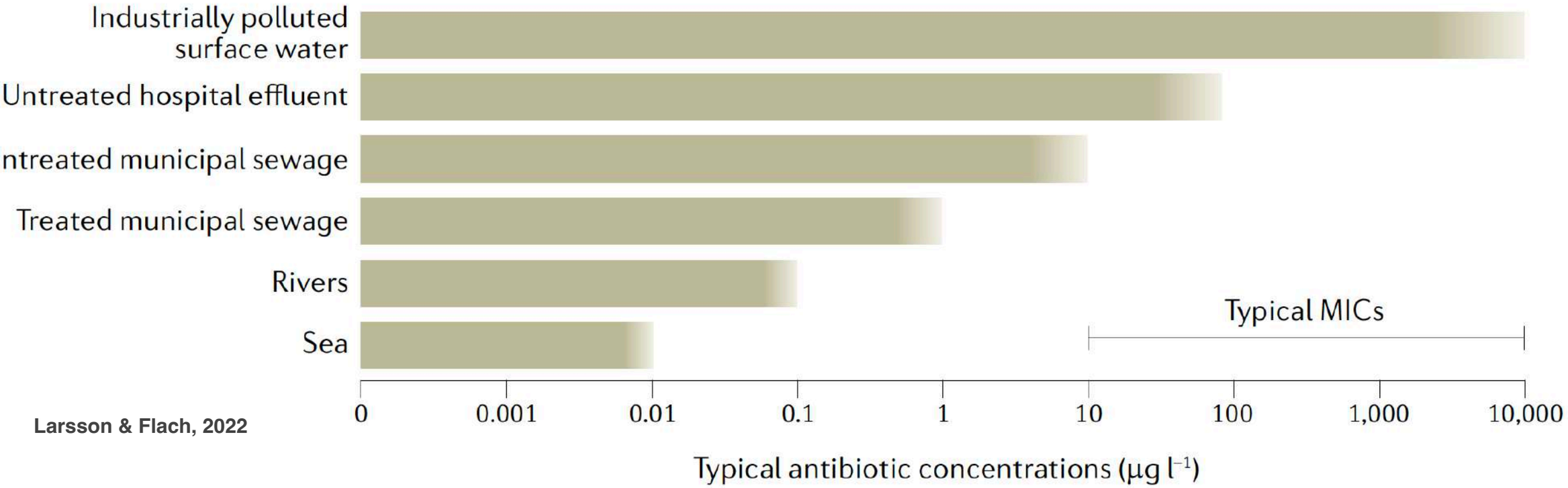
Morens & Fauci, 2020

AMR, AntiMicrobial Resistance
ARGs, Antimicrobial Resistance Genes
Antibiotic crisis

Antibiotic occurrence in our society

- Hospital
- At home (supervised or not supervised)
- Agriculture
- Chemical and pharmaceutical Industry
- Farming
- Wastewater treatment plants

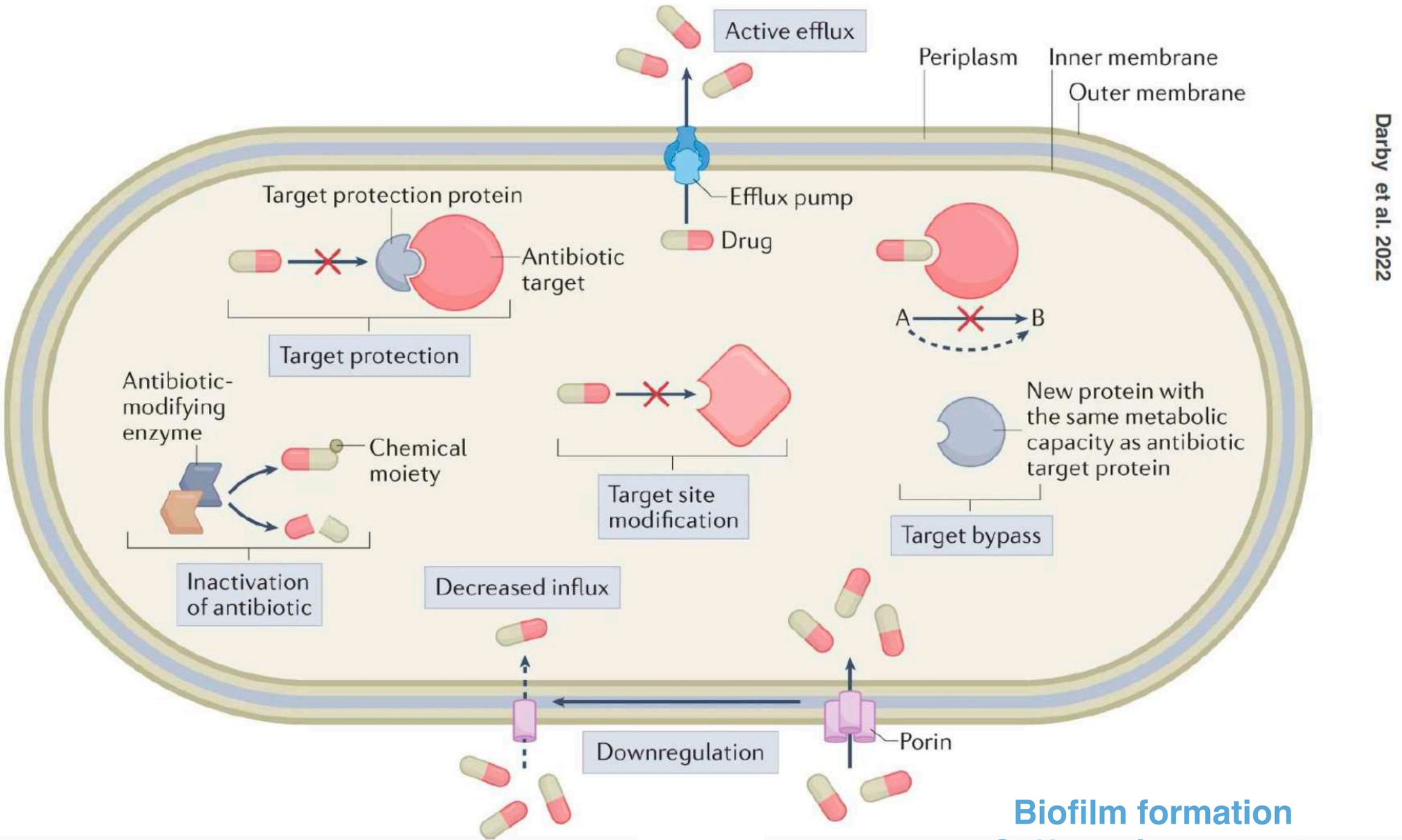
Antibiotic concentrations in the environment



Larsson & Flach, 2022

Typical minimal inhibitory concentrations (MICs) for many antibiotic–pathogen combinations often fall within the 10–10000 μg L^{-1} range

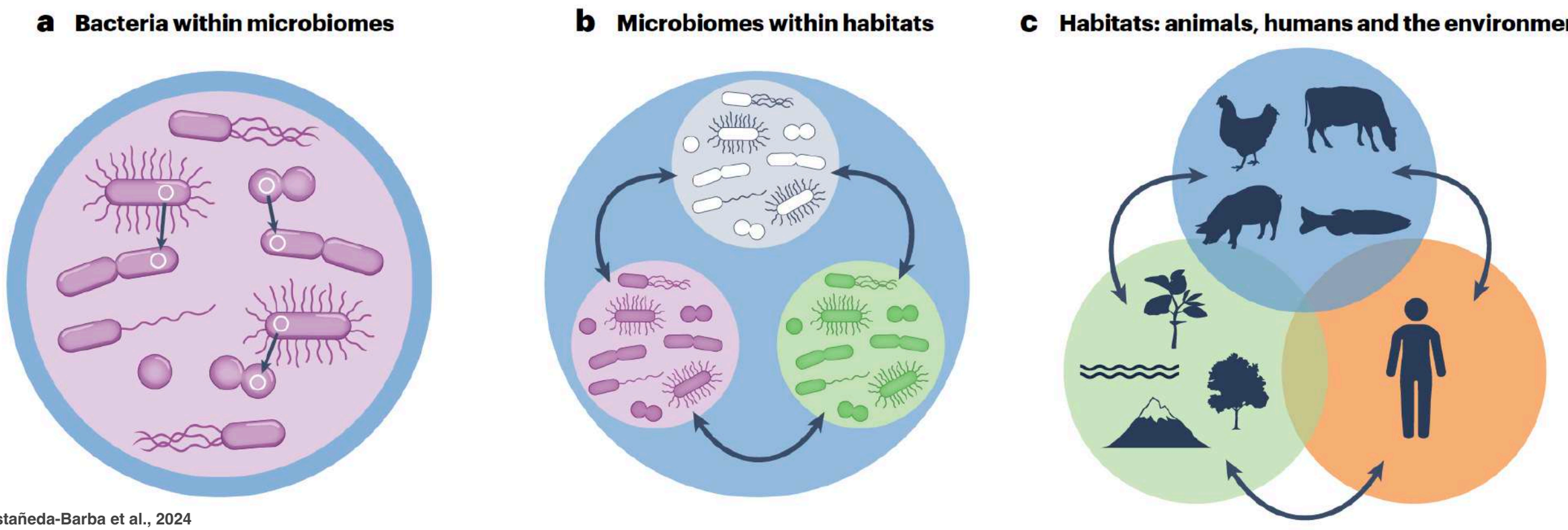
Antibiotic Resistance mechanisms



Darby et al. 2022

Biofilm formation
Shift to slow growth
Shift to stress response activation pathways
Persistance

The emergence of antibiotic-resistant bacteria is due, in large part, to the spread of antimicrobial resistance genes via horizontal gene transfer and mutations

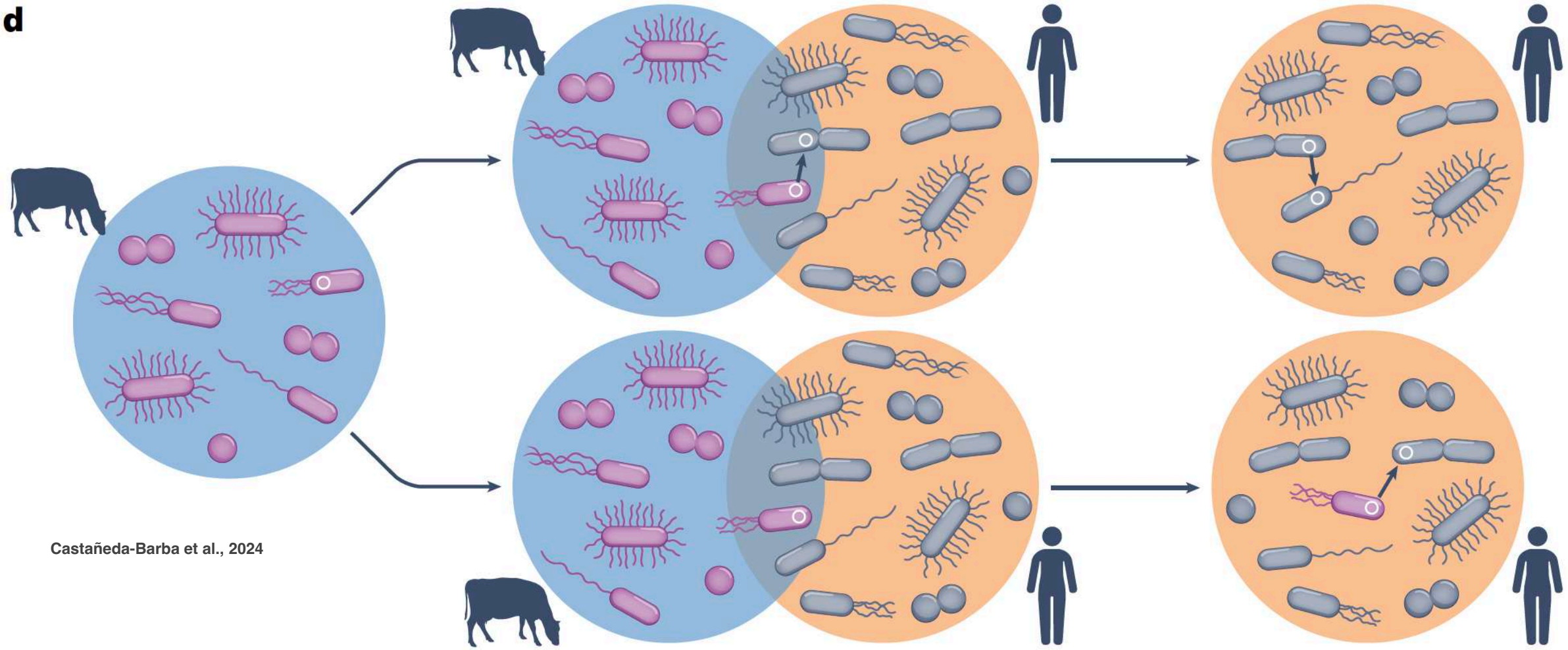


Castañeda-Barba et al., 2024

- **Plasmids** function as vehicles that transfer antimicrobial resistance genes **between** bacteria, including **distantly related species**

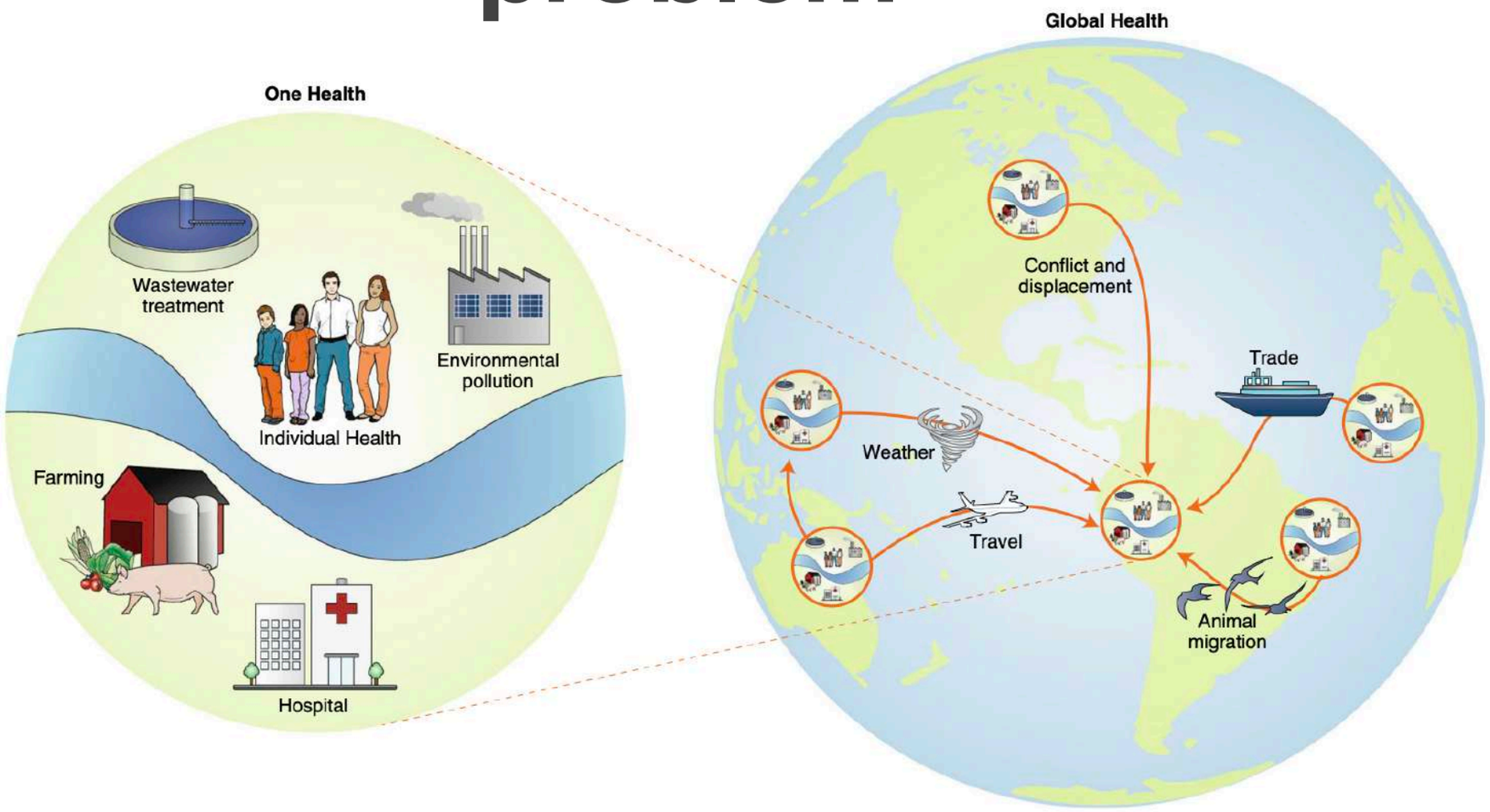
- In contrast, **insertion sequences, transposable elements** promote **gene exchange within cells**

Antibiotic resistance gene spread in a One Health framework



- A hypothetical pathway of a resistance plasmid between habitats
- **The spread of a plasmid can occur at areas of confluence, where microbiomes of two habitats overlap**
- An example of such an area of confluence in farm settings arises when workers interact with cows
- In this interaction, a plasmid can spread directly between animal and human bacteria, or bacteria from the cow microbiome can survive long enough in the human microbiome to subsequently spread their plasmid

AMR is One Health and Global Health problem



Hernando-Amado1 et al., 2019

- **AMR transmission** occurs at the **local level across the borders between different ecosystems:** farms, hospitals, wastewater treatment plants and natural environments
- **One Health is a 'local version' of Global Health, which addresses communication among local ecosystems and the global conditions that facilitate the worldwide spread of AMR**
- Global interchange of goods by human travellers, migrating animals and even through the help of natural phenomena
- **Corridors and bridges therefore exist that promote the globalization of gene spread**, encouraging the appearance of similar microbial communities wherever the same processes occur