





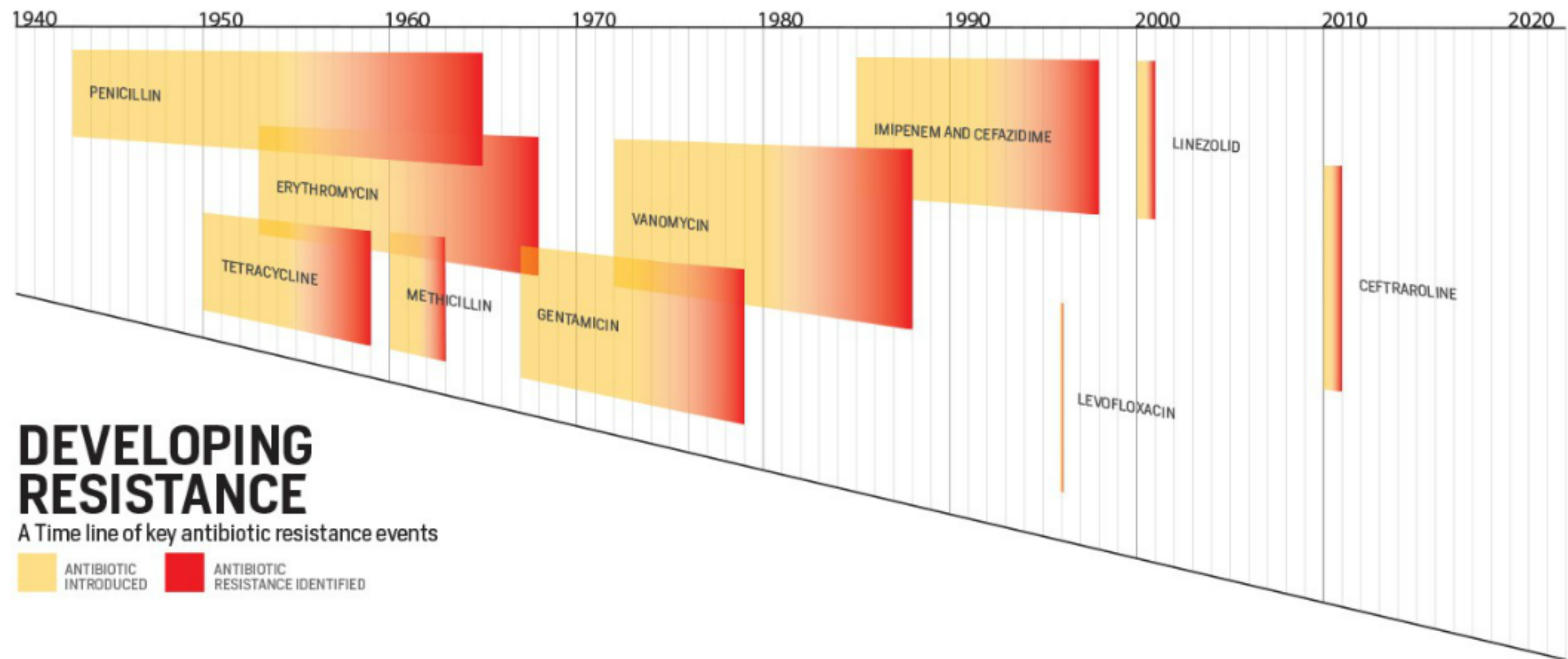




Antimicrobial Resistance

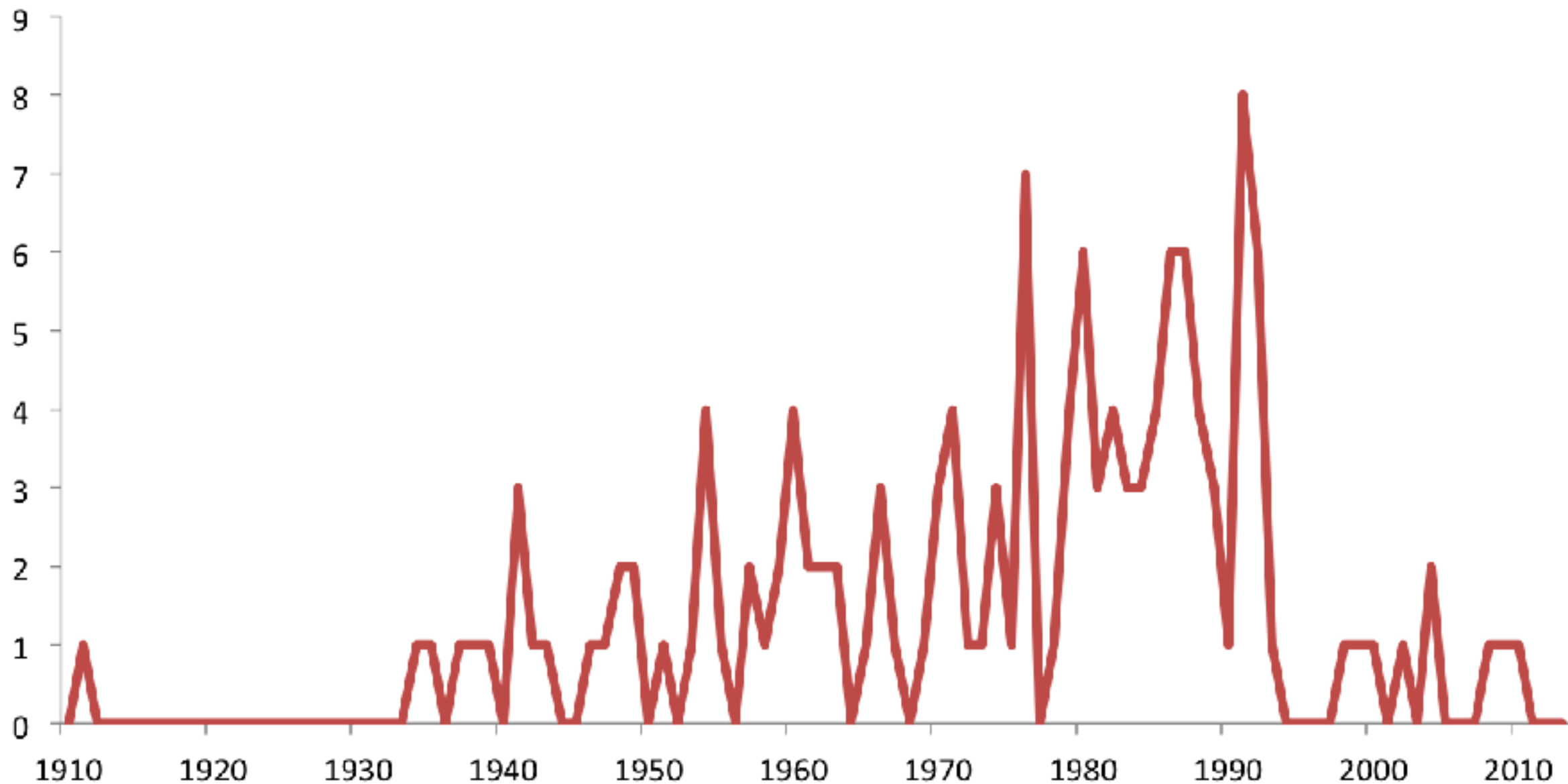


Antimicrobial Resistance is on the Rise



We Have Reached "Peak Antibiotics"

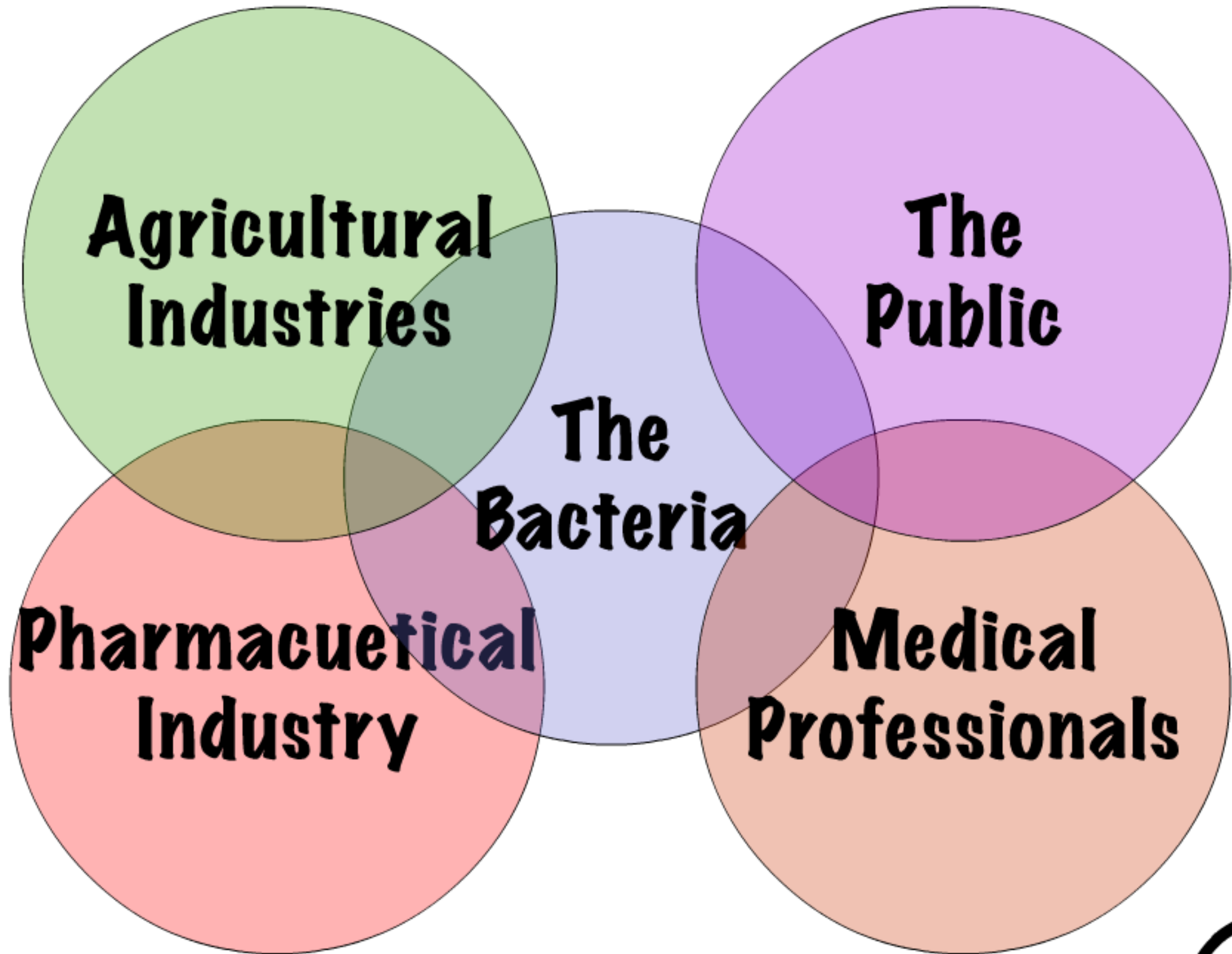
Antibiotics to market per year



On average antibiotics add 20 years to each person's life.



The Players in Antibiotic Resistance



Questions:

Bacteria, a microbiologists perspective.

What are antimicrobials ?

Where does antimicrobial resistance come from?

Do antimicrobial products matter?

How bad is the problem is it?

What can we do?



Bacteria

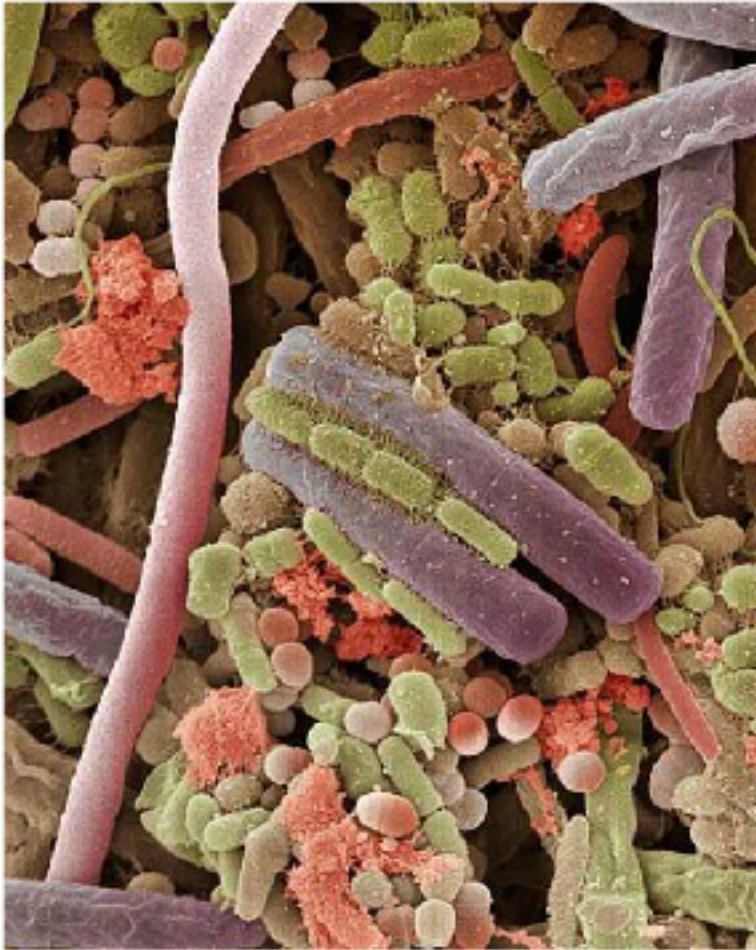
single cell
microscopic
organisms



grow
and divide
into two



Bacterial Communities are Everywhere



Mixed : Human tongue



Mixed : Dental plaque



Serratia : bathroom

Complex and Interdependent

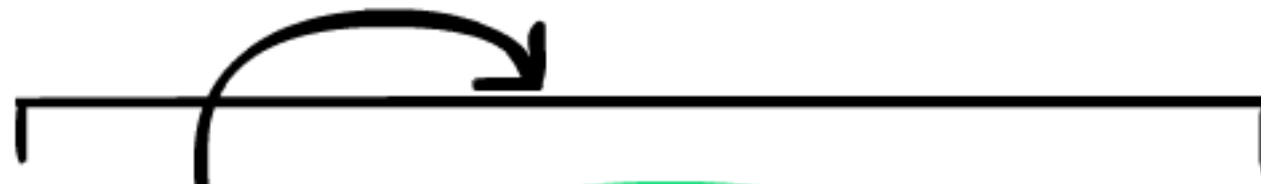


Antimicrobial Resistance



Hendrickson

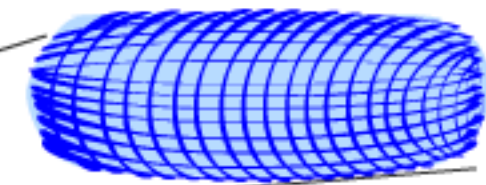
How small is small? 2 microns long



Human Hair .0889 mm



**1 Micron =
0.001mm**



2 Microns



Some Bacteria Grow and Divide in 20 Minutes!



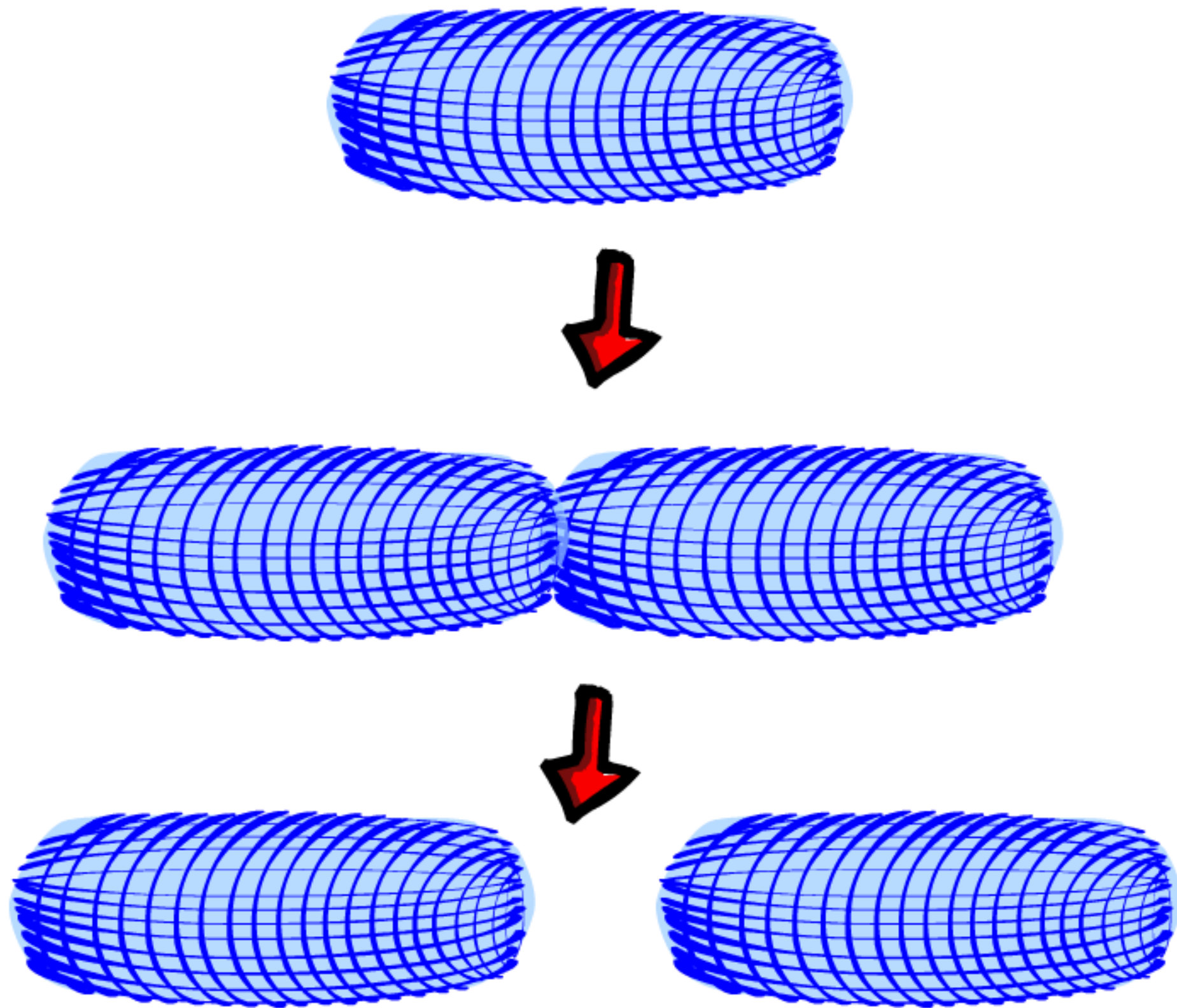
8 hours - movie courtesy of Dr. Christian Lesterlin

Antimicrobial Resistance

Hendrickson



Bacterial Reproduction is Clonal



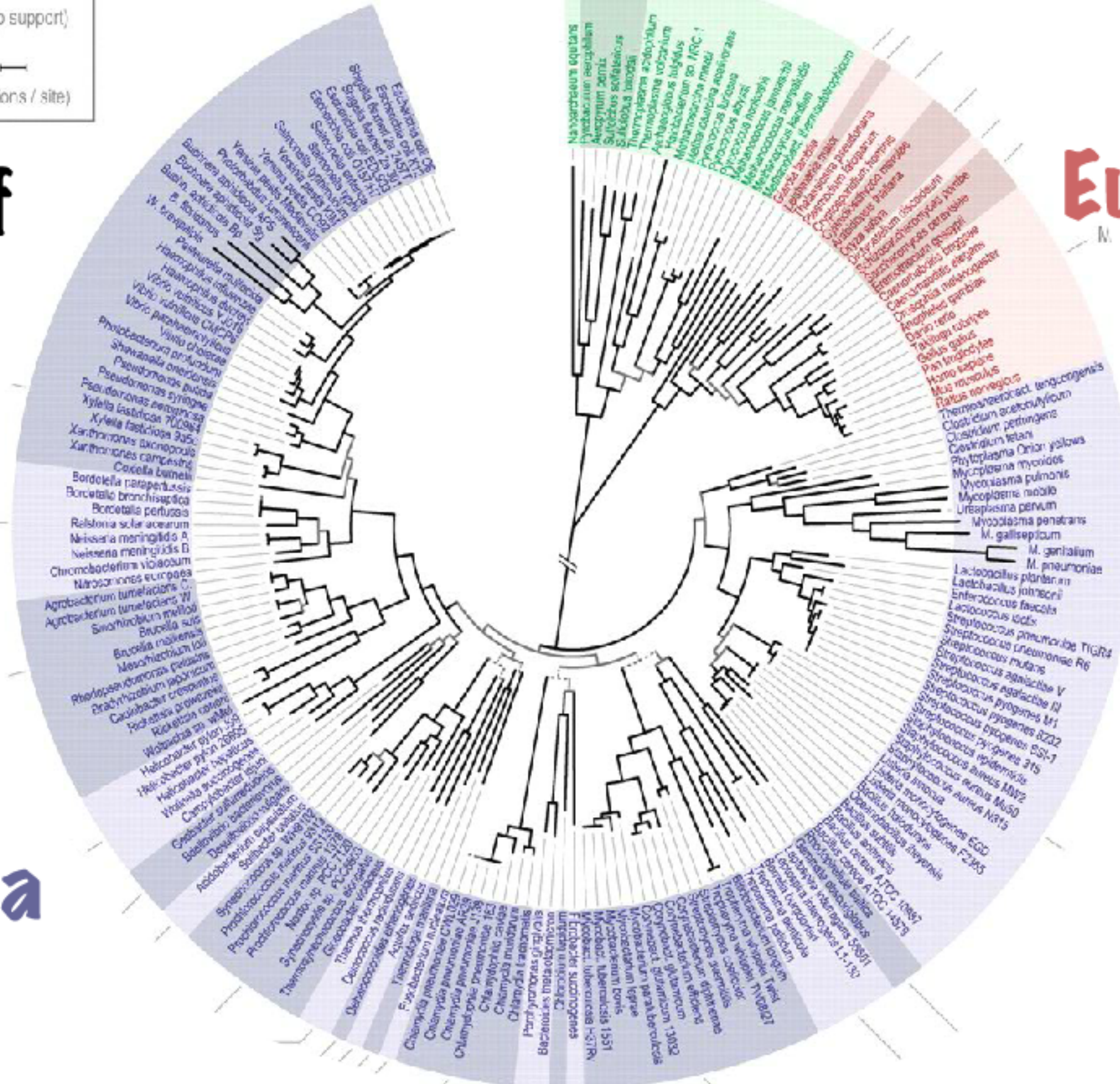
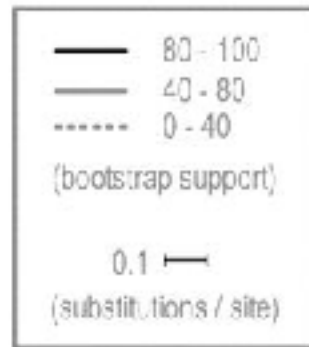
Does not depend on recombination (sex).



Archaea

Eukaryotes

Tree of Life



Bacteria



Antimicrobial Resistance

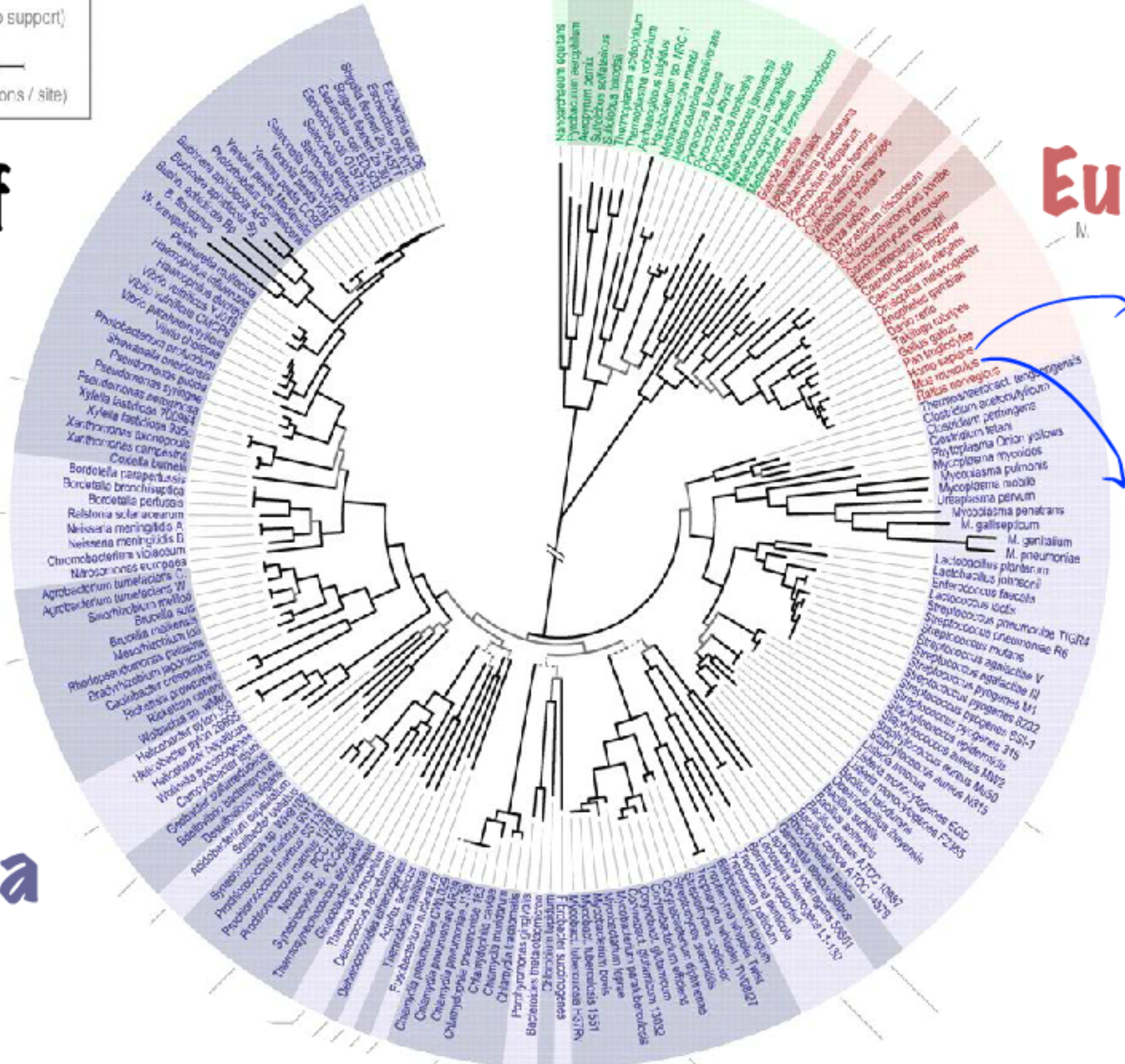
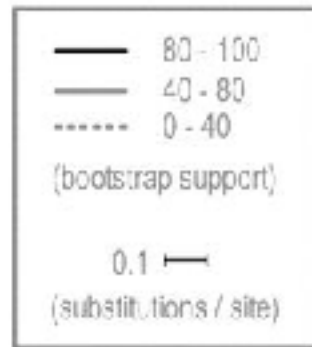
Hendrickson



Archaea

Eukaryotes

Tree of Life



Bacteria

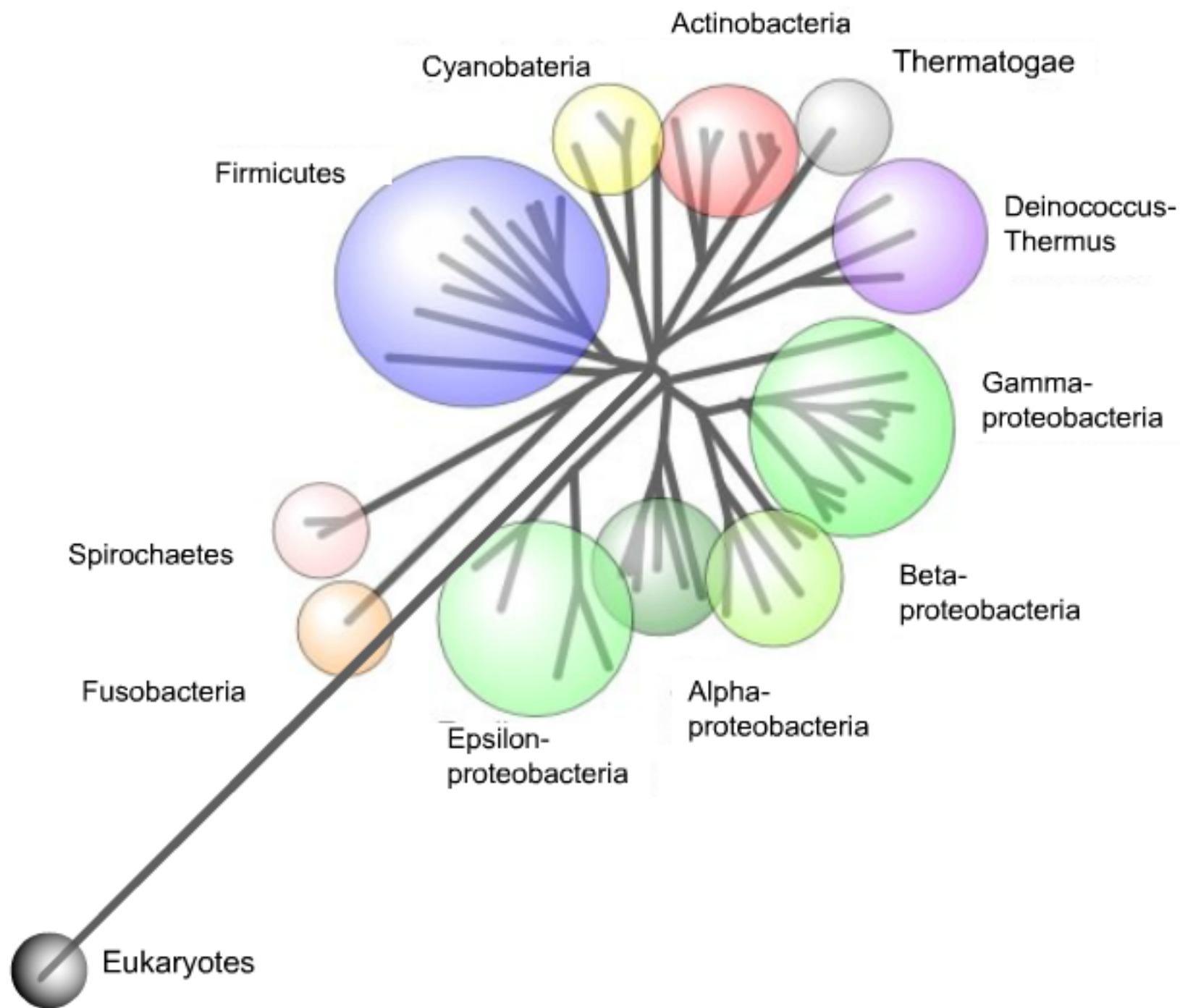


Antimicrobial Resistance

Hendrickson



Bacteria in Perspective



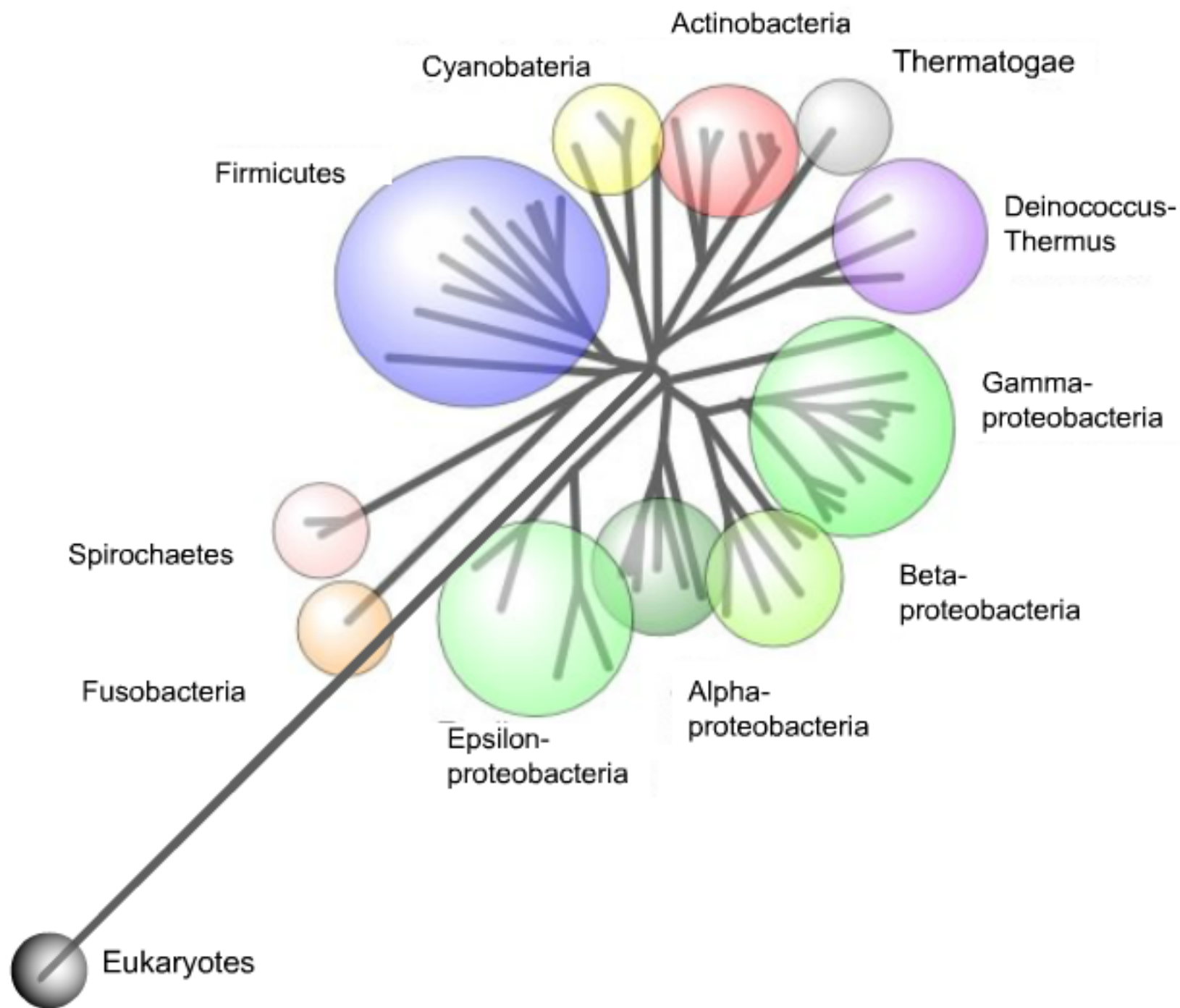
Genetically and physiologically diverse

The majority of planetary biomass

The majority of chemistry



Bacteria in Perspective



Genetically and physiologically diverse

The majority of planetary biomass

The majority of chemistry

Fix nitrogen from air

Do photosynthesis

Follow geomagnetic fields

Glow in the dark

Live in nuclear power plants

Eat oil

Cheat

Talk to one another

Make us happy

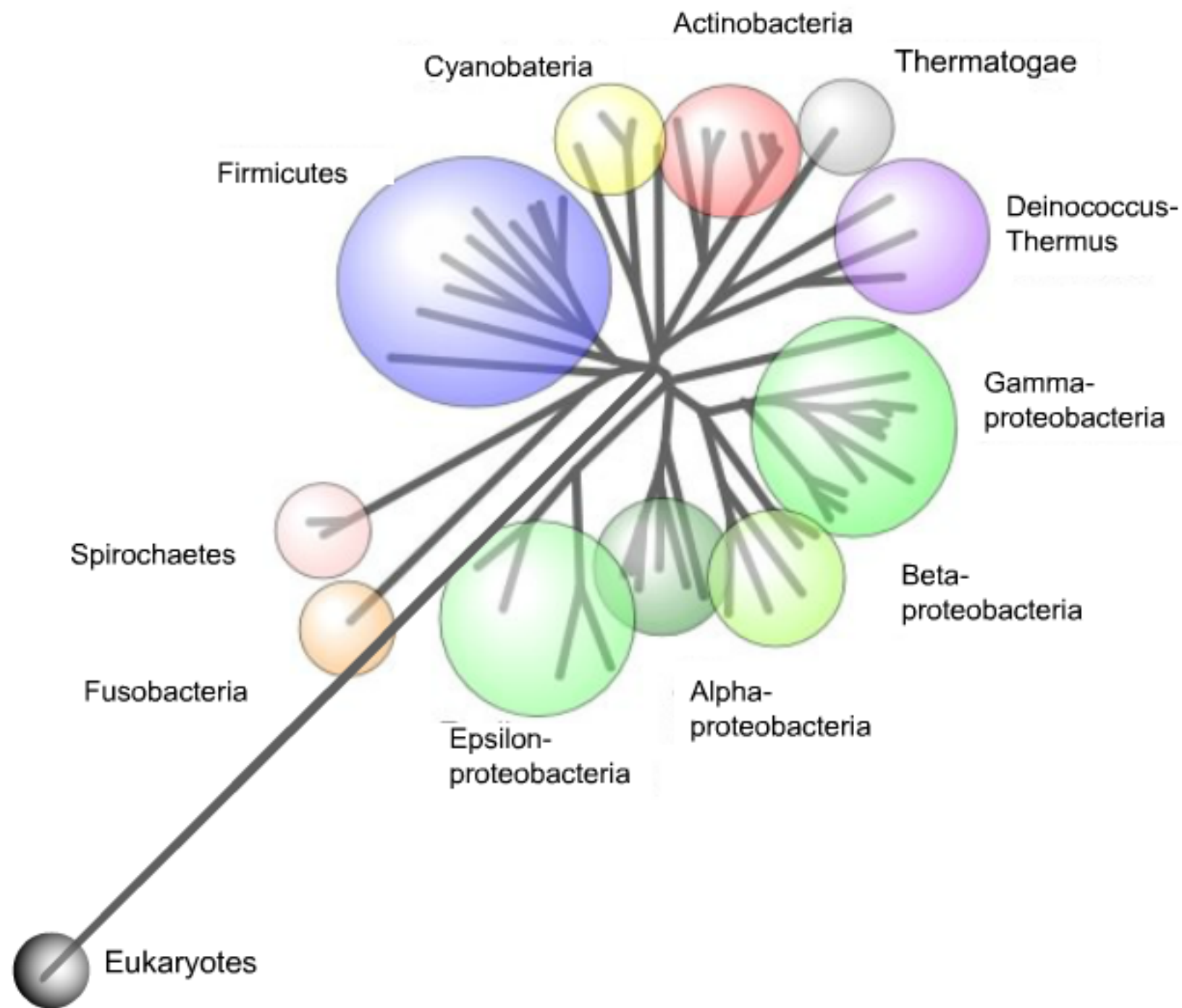


Antimicrobial Resistance

Hendrickson



Bacteria in Perspective



cause disease

Genetically and physiologically diverse

The majority of planetary biomass

The majority of chemistry

Fix nitrogen from air

Do photosynthesis

Follow geomagnetic fields

Glow in the dark

Live in nuclear power plants

Eat oil

Cheat

Talk to one another

Make us happy



Antimicrobial Resistance

Hendrickson



>99% Unknown



You are made out of Bacteria

**Only 10% of cells
in a human
are human cells...**

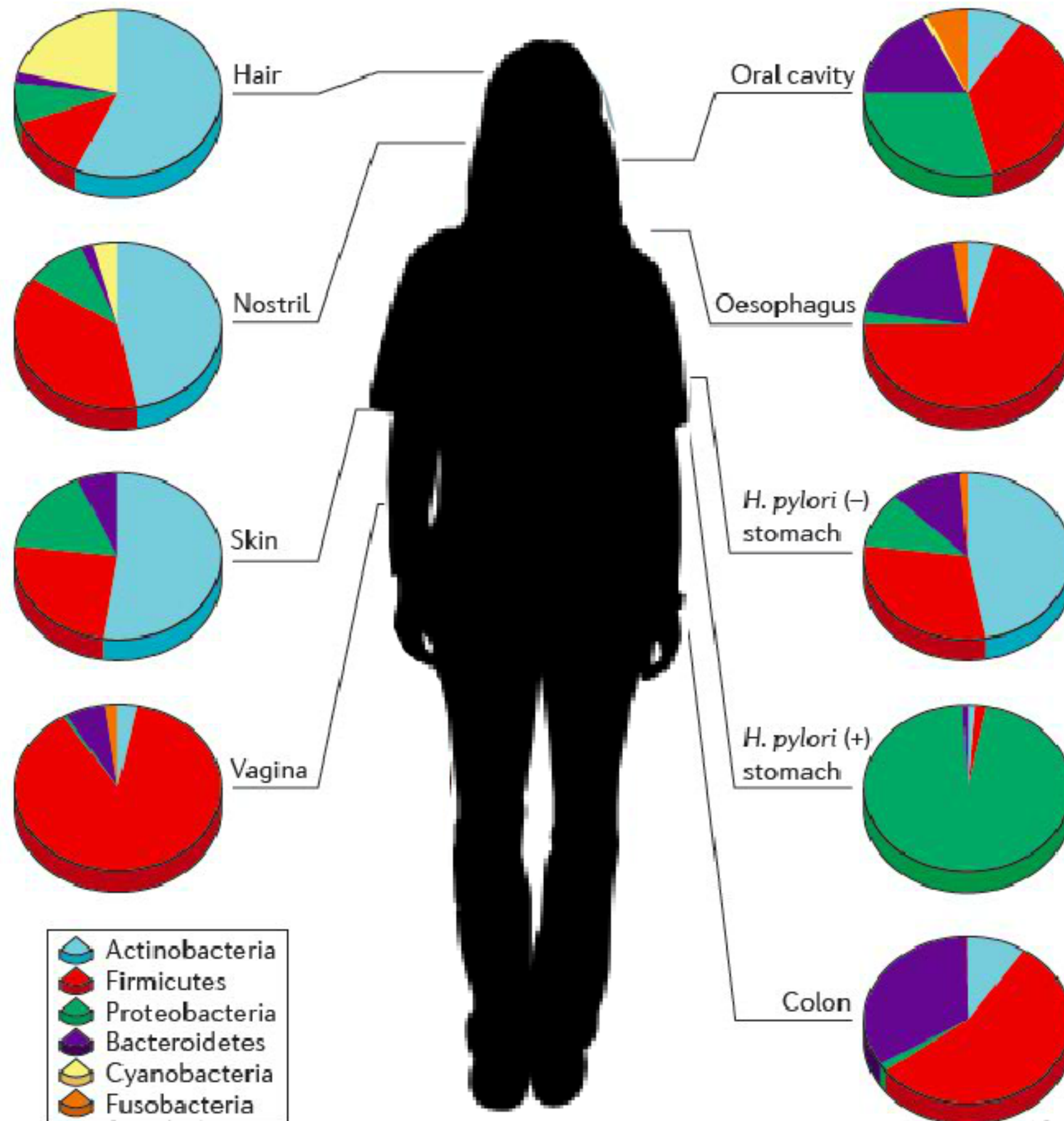


**1 trillion
human cells**

**10 trillion
bacterial cells**



What are they doing?



What are they doing?

**Some crowd
out potential
pathogens.**



**Basically they
are just selfish
entities...**



Where do antibiotics come from?



Antimicrobial Resistance

Hendrickson



Where do antibiotics come from?

1) Pharmacist / doctor

2) Pharmaceutical companies

3) Microorganisms



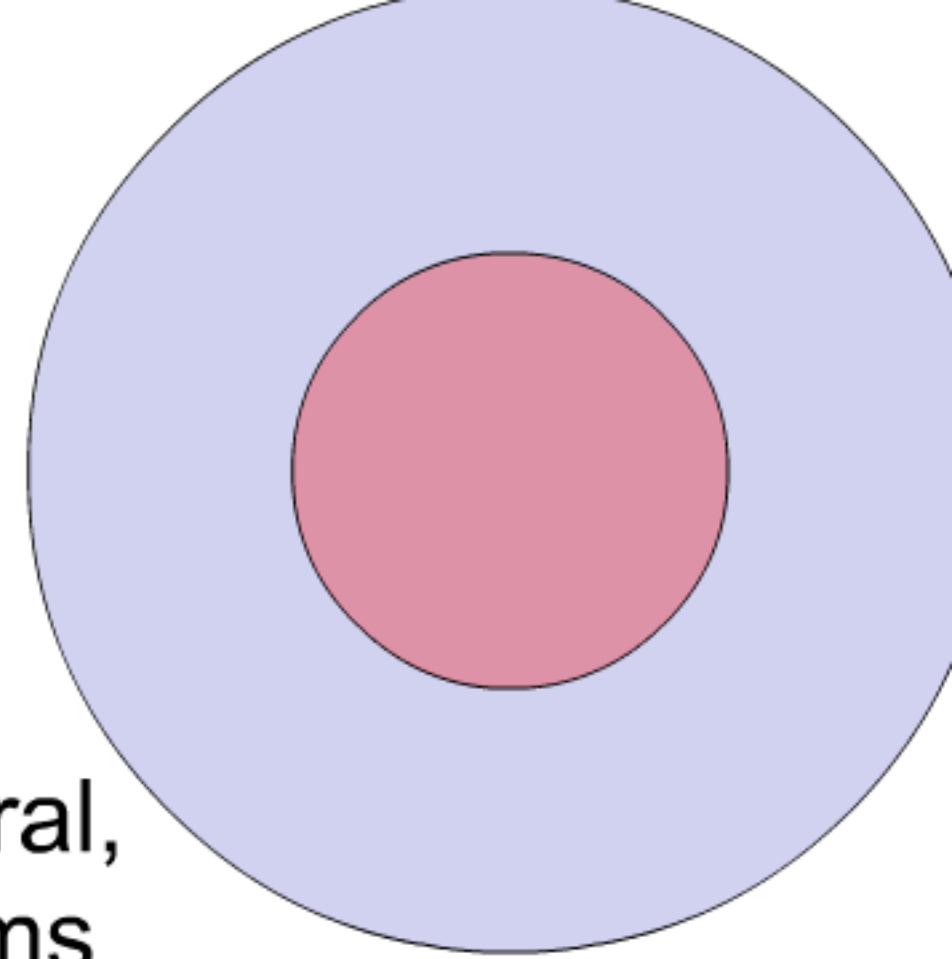
Some Vocabulary...

**Antimicrobials =
against little life**

-any substance, synthetic or natural,
that inhibits or kills microorganisms.

**Antibiotics =
(life) against life**

-natural substances made by microorganisms
that inhibit or kill other microorganisms.



A good antimicrobial will be:

Non-toxic to animals

Kill or stop bacteria
(bacteriocidal or bacteriostatic)

Easy to produce

Affect some or all bacteria
(narrow vs. broad)

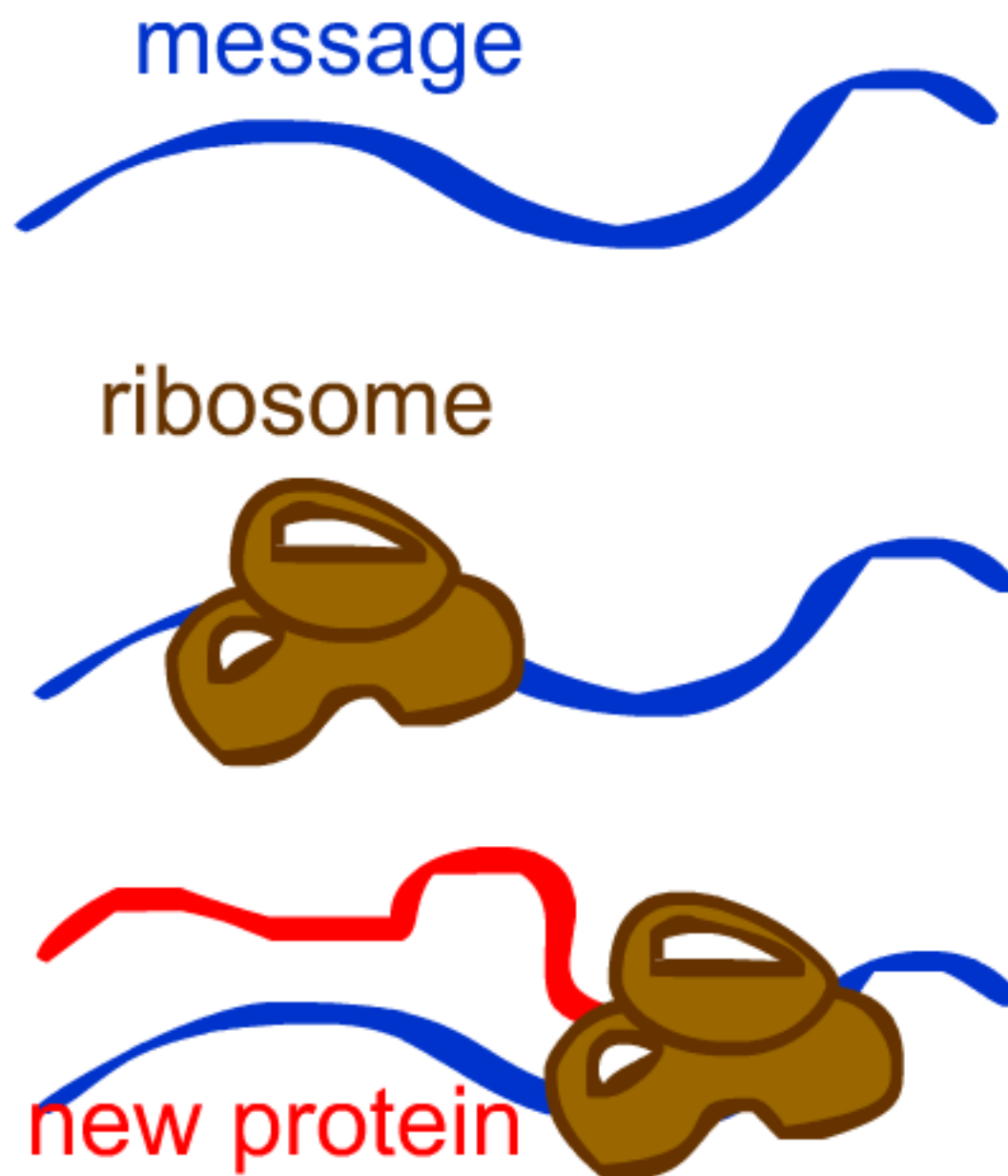


Examples of antimicrobials and resistance mechanisms



Bacteriostatic Antimicrobials:

Examples:



An antibiotic that interferes with protein production

No new proteins mean
No new DNA
No reproduction....
Cell **stasis**.



Bacteriostatic Antimicrobials:

Examples: tetracycline

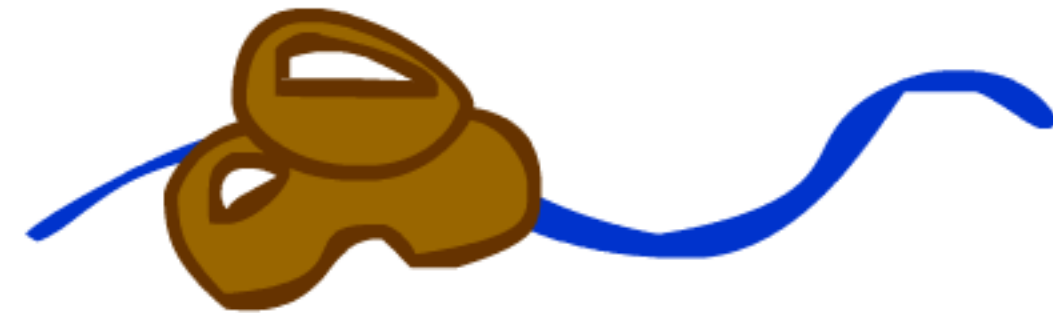
Broad spectrum antimicrobial
Interferes with protein production



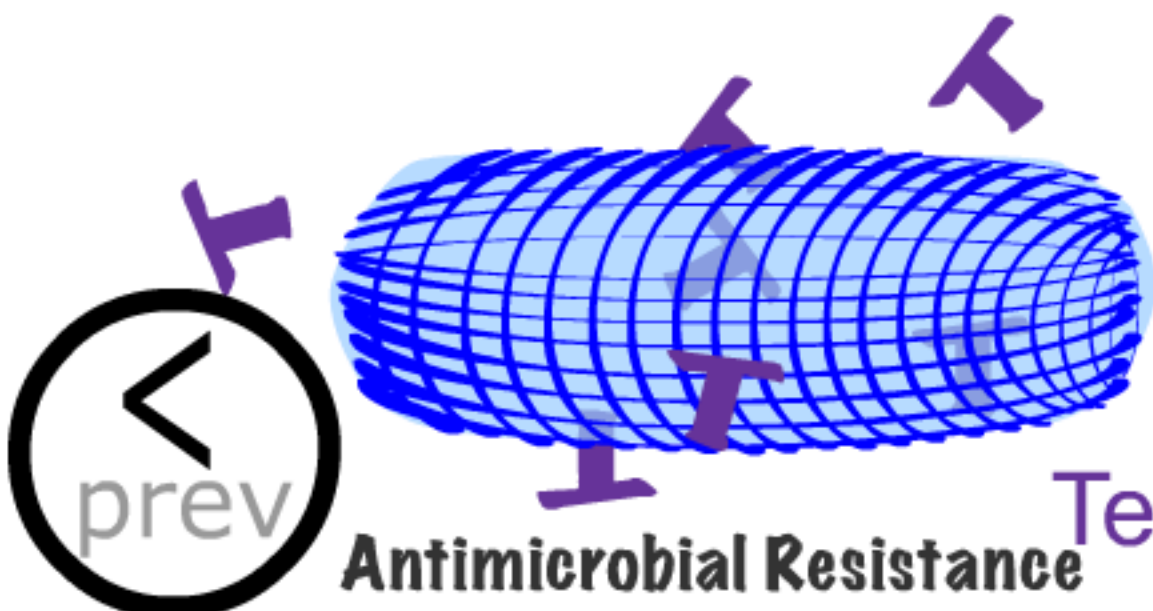
message



ribosome



new protein



Antimicrobial Resistance

Tetracycline stops ribosome

Hendrickson



Bacteriostatic Antimicrobials:

tetracycline resistance genes:

tetracycline efflux



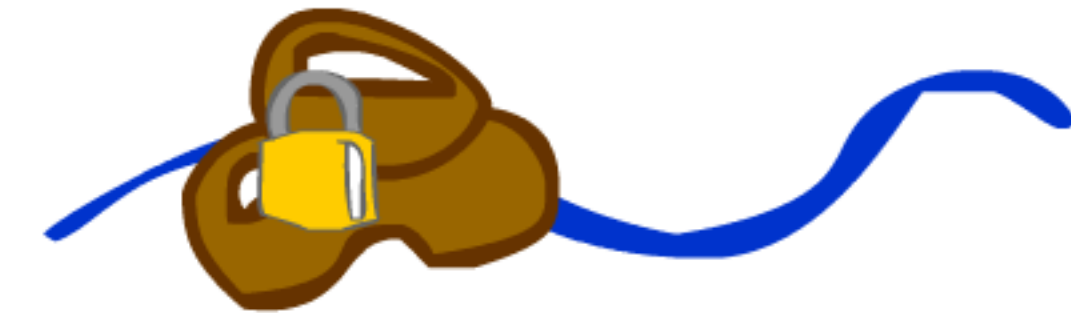
ribosome protection



message



ribosome



tetracycline modification



new protein



Tetracycline stops ribosome

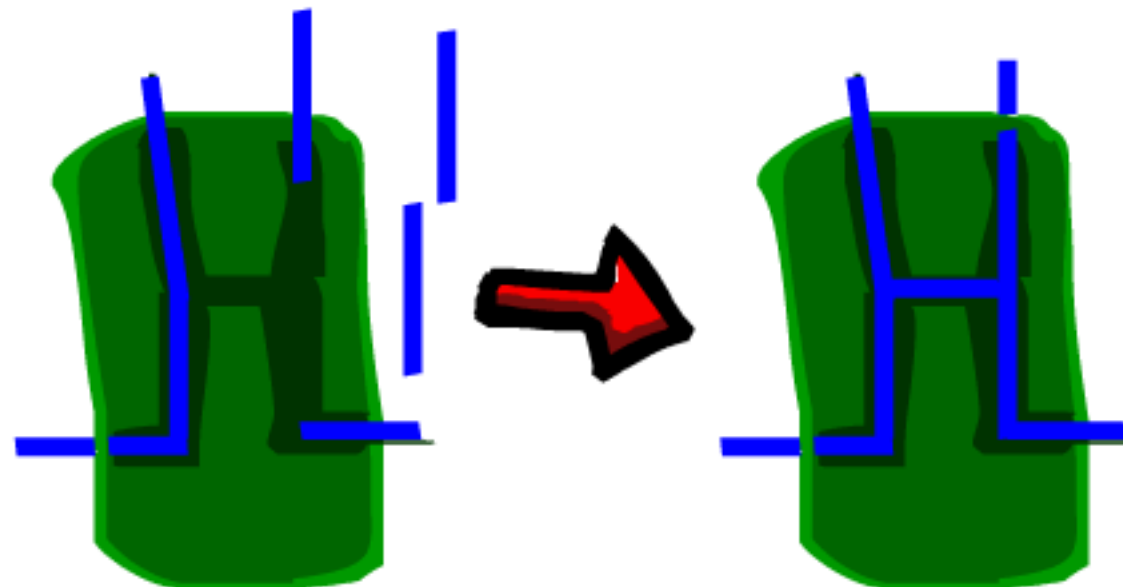
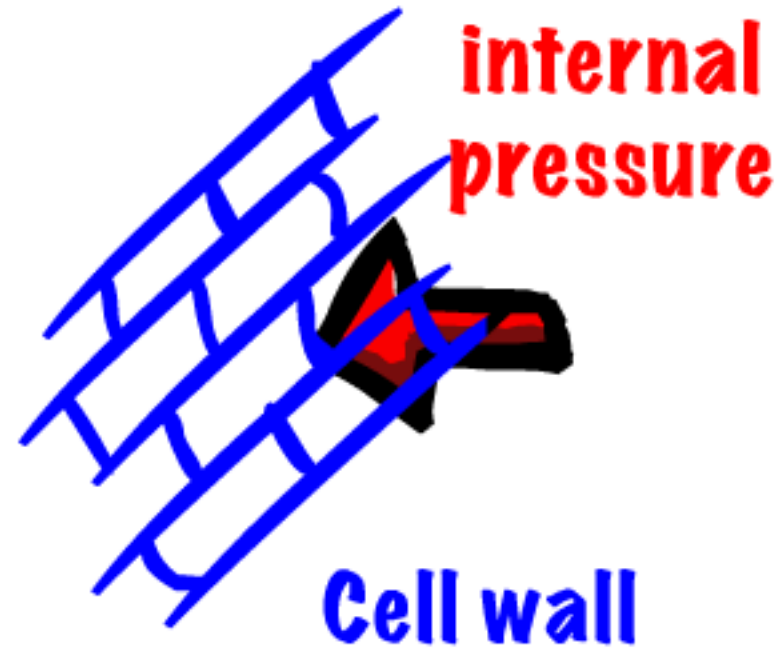
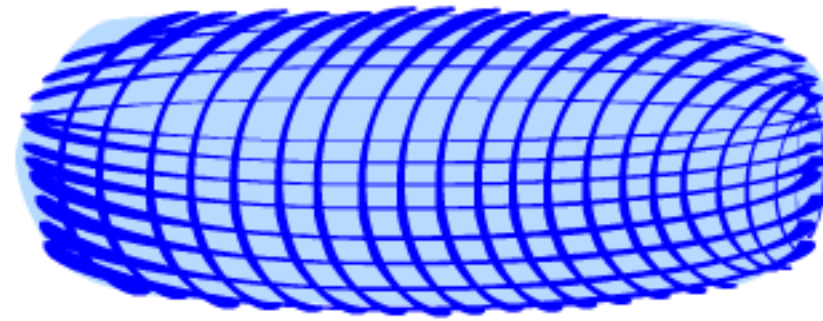


Hendrickson



Antibiotic Resistance

Bacteriocidal Antimicrobials:

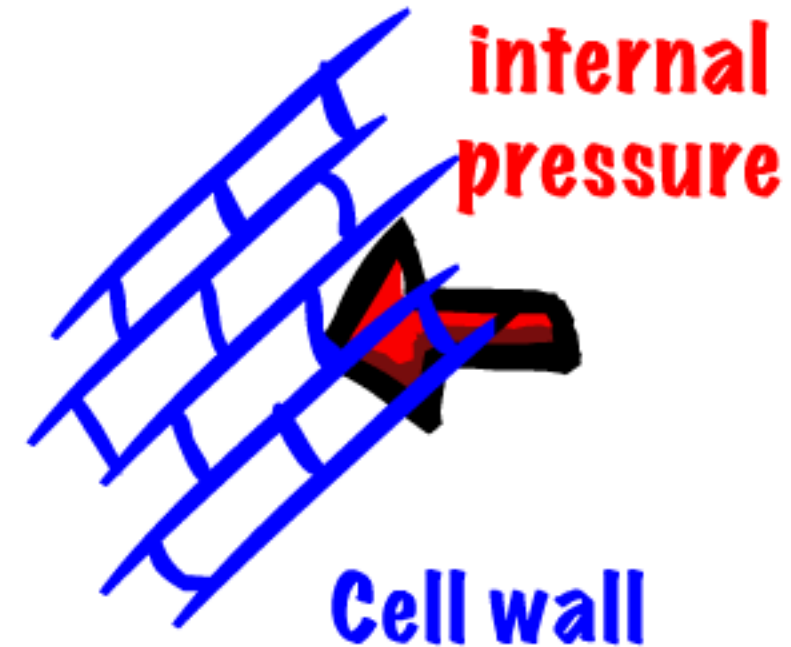
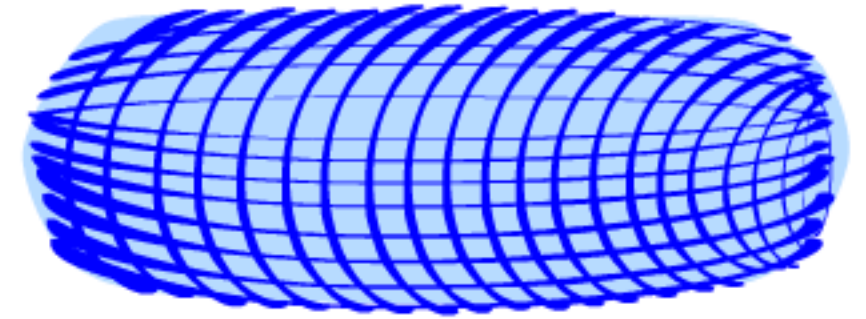


Bacteriocidal Antimicrobials:

Examples: beta-lactams

Most widely used group of antibiotics:

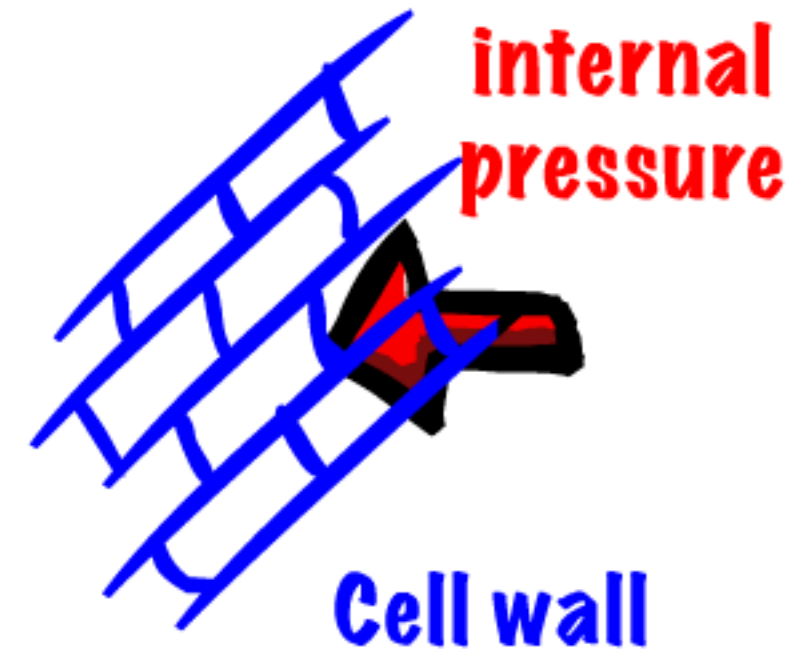
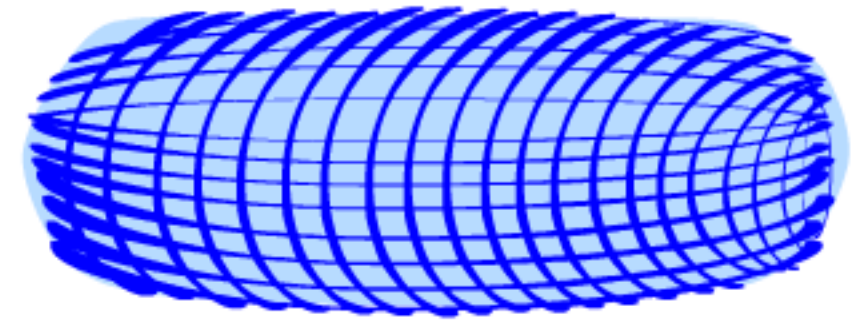
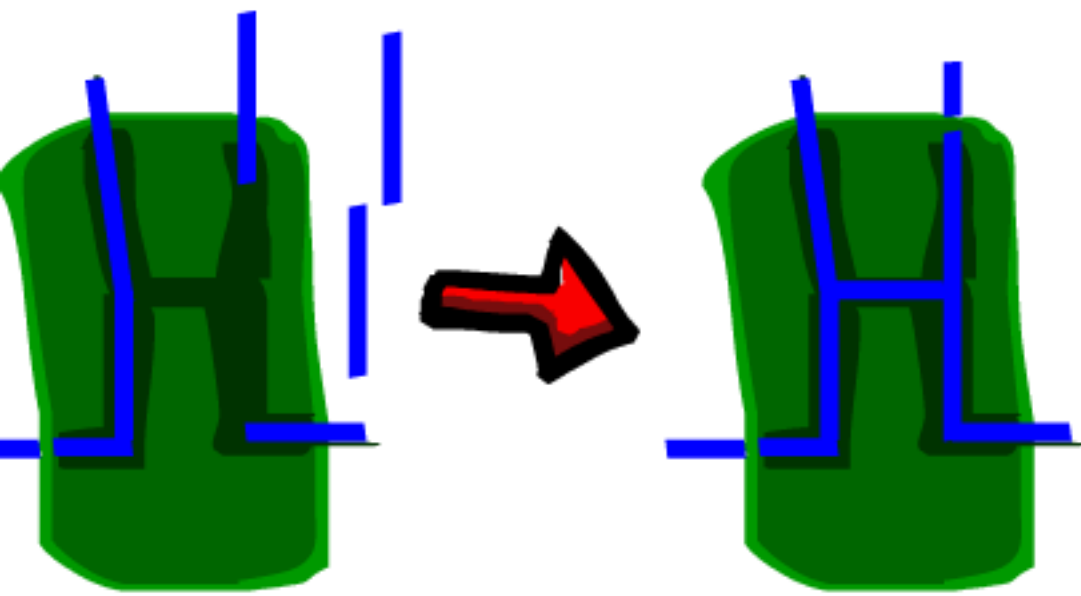
- ~ Penicillin
- ~ Ampicillin
- ~ Cephalosporins
- ~ Methicillin



Bacteriocidal Antimicrobials:

Examples: beta-lactams

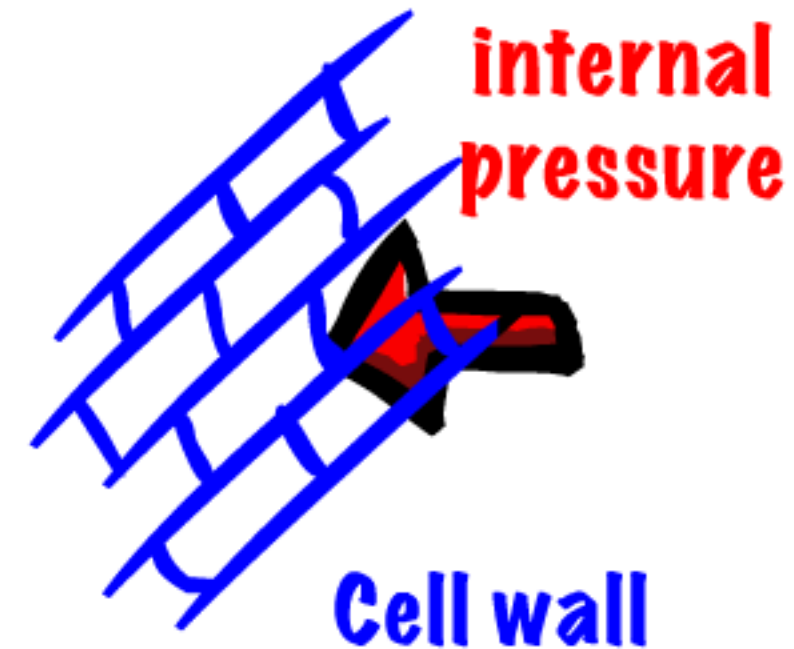
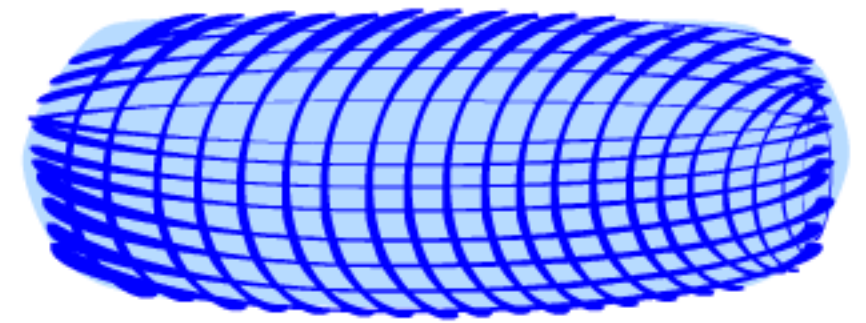
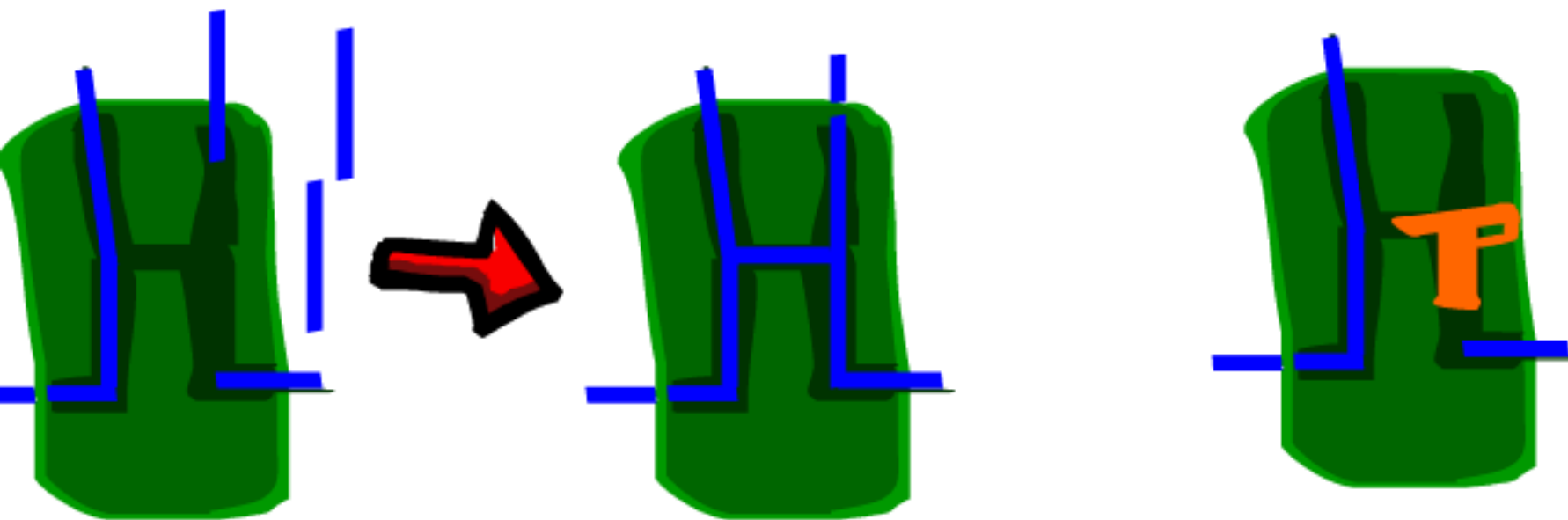
Inhibit the cell wall building enzymes:



Bacteriocidal Antimicrobials:

Examples: beta-lactams

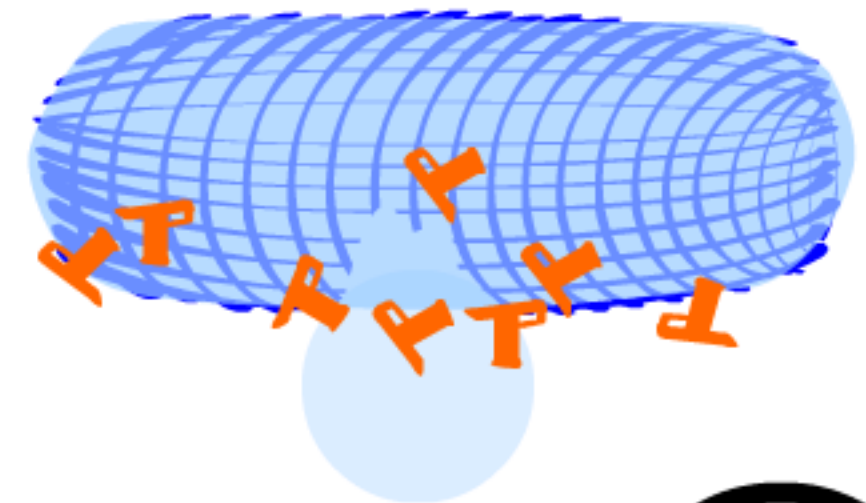
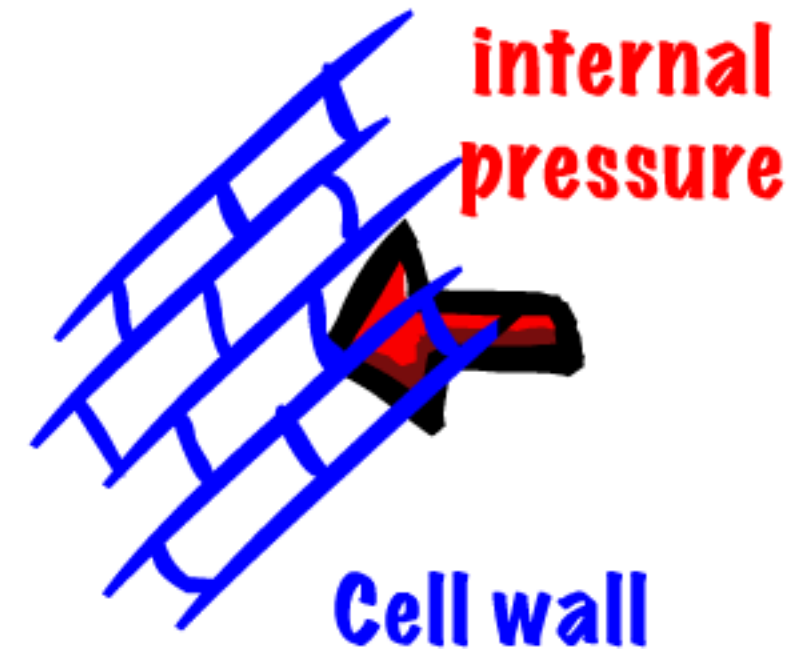
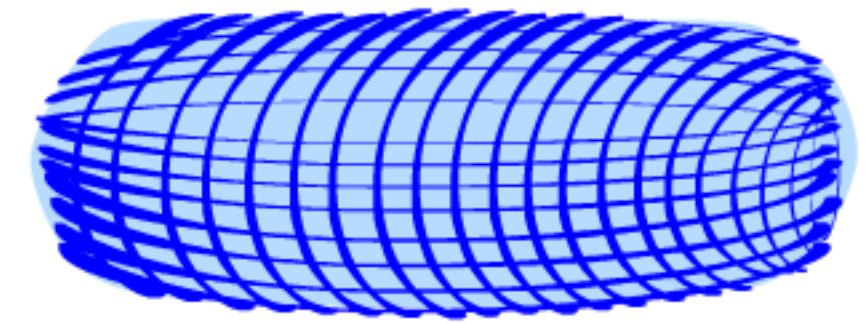
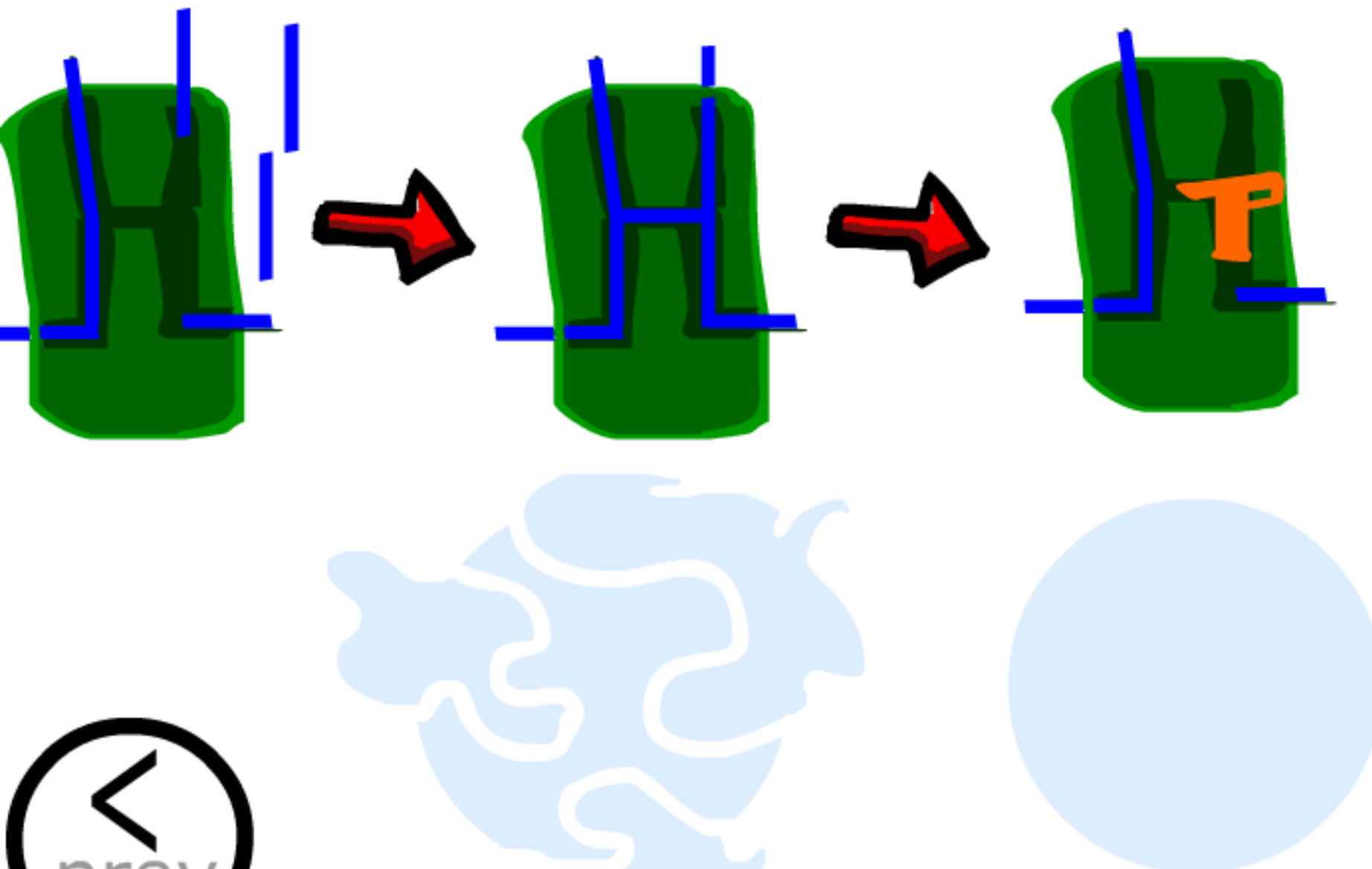
Inhibit the cell wall building enzymes:



Bacteriocidal Antimicrobials:

Examples: beta-lactams

Inhibit the cell wall building enzymes:



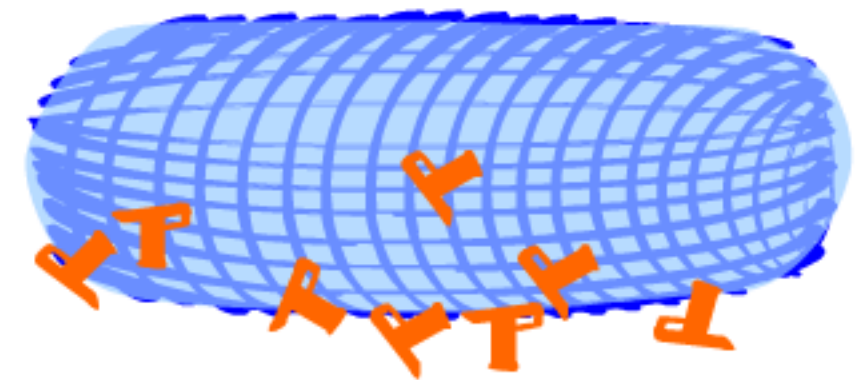
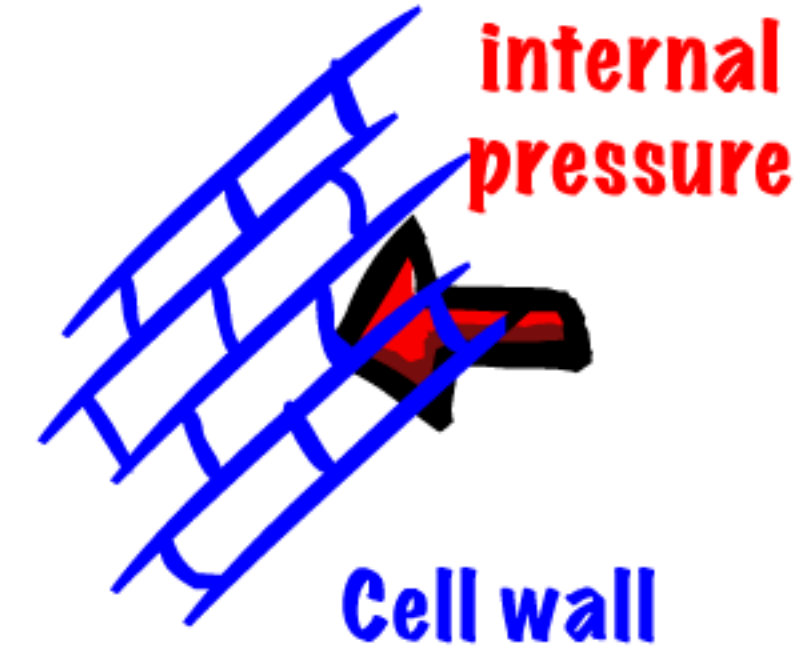
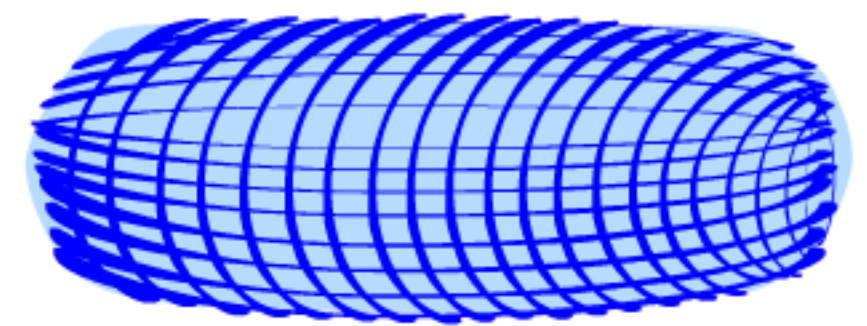
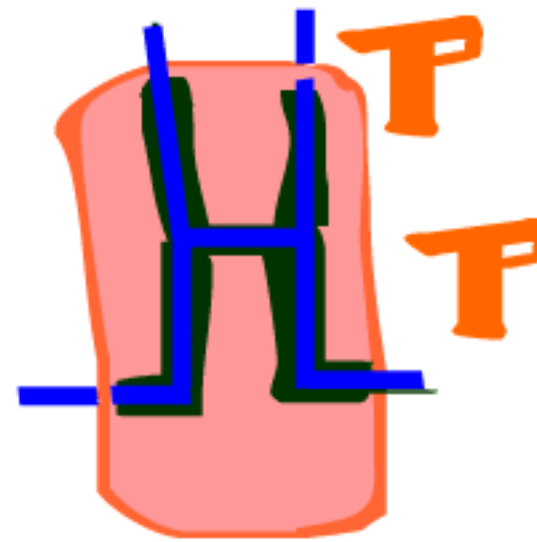
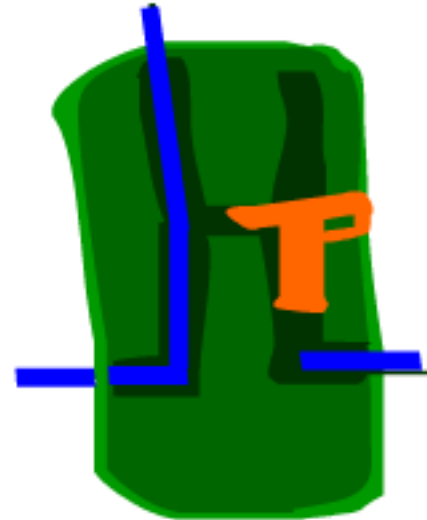
Beta-lactam resistance genes

Cell wall building enzyme:

Penicillin binds to inhibit.

New cell wall enzyme

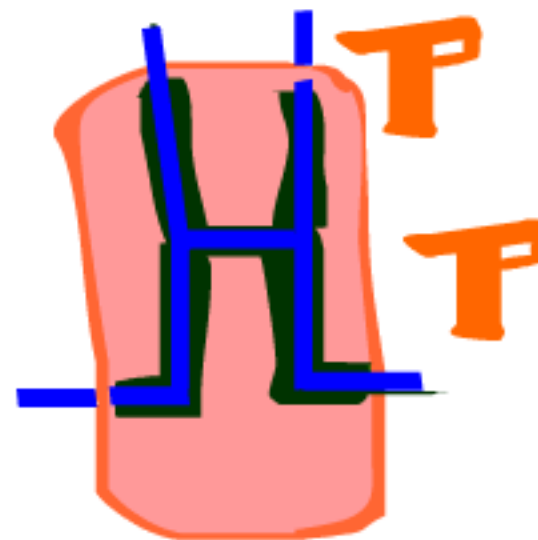
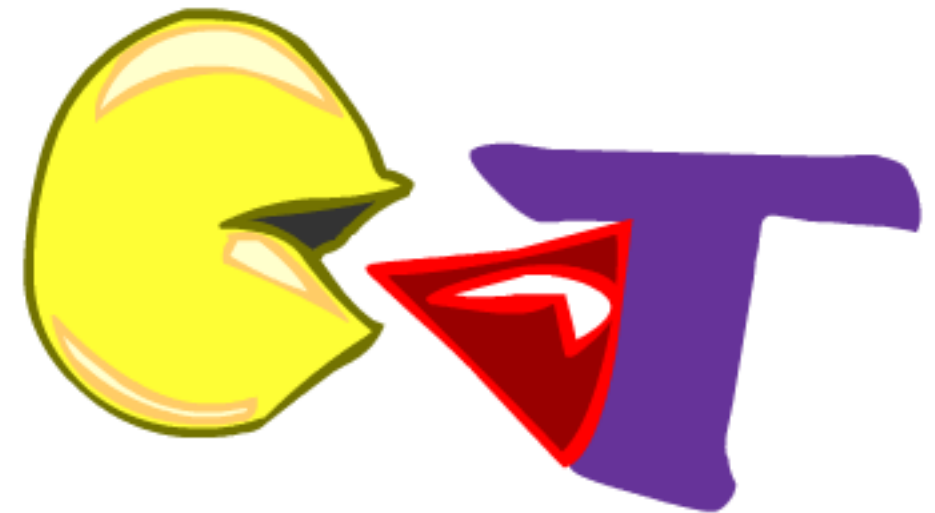
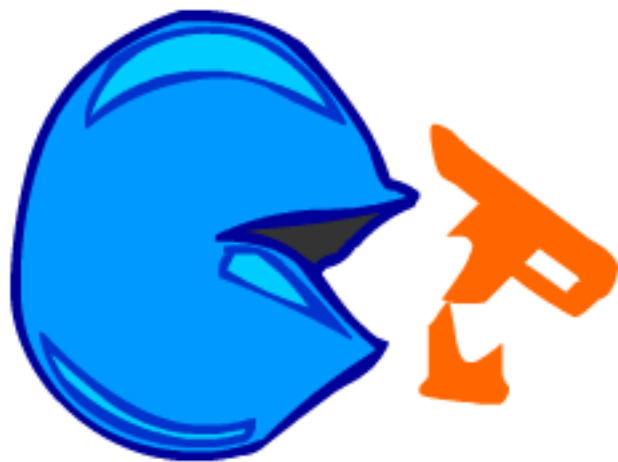
Beta lactamase (eater)



How is resistance evolving?



4 Major Mechanisms



Antibiotic Resistance

Hendrickson

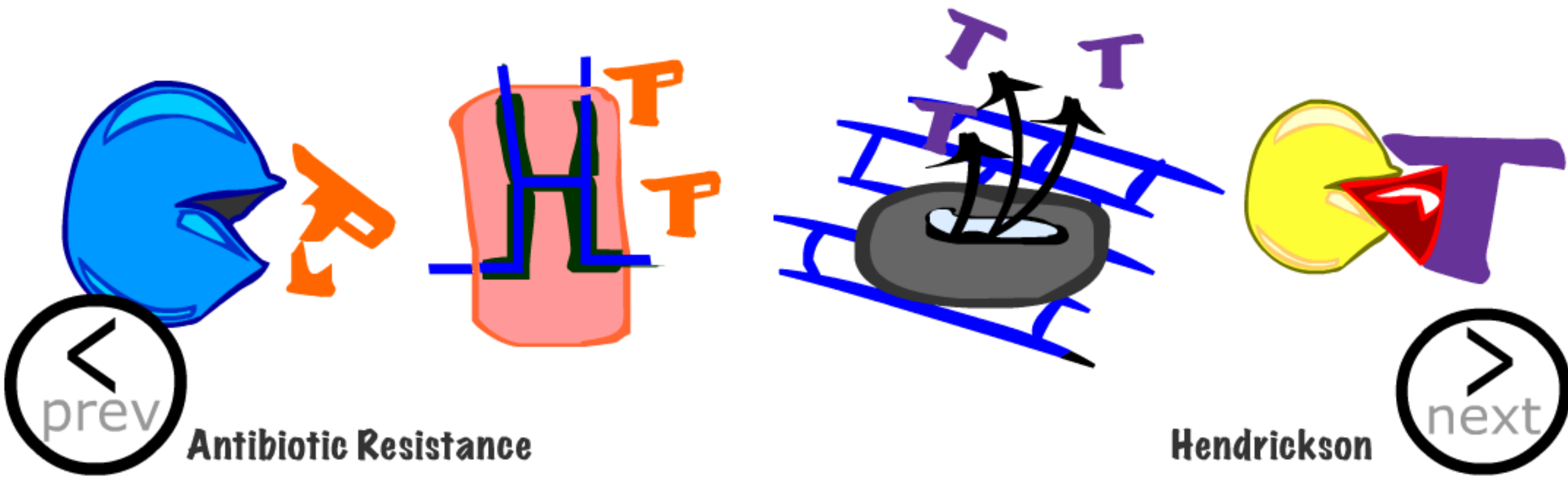


How is resistance evolving?

Enzymes evolved in the presence of antibiotics.

These enzymes are encoded for by genes in DNA.

Genetic material in bacteria is chimeric.



Bacteria are Genetic Chimeras

"a thing of immortal make,
not human, lion-fronted
and snake behind, a goat
in the middle, and
snorting out the breath of
the terrible flame of bright
fire"

~Homer (Iliad)



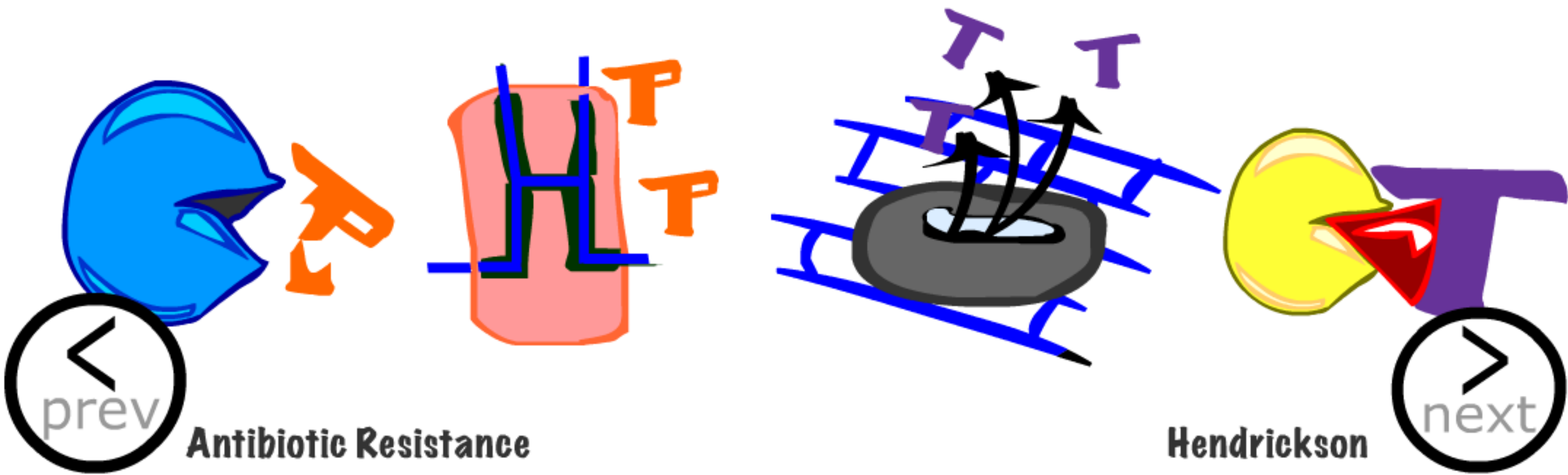
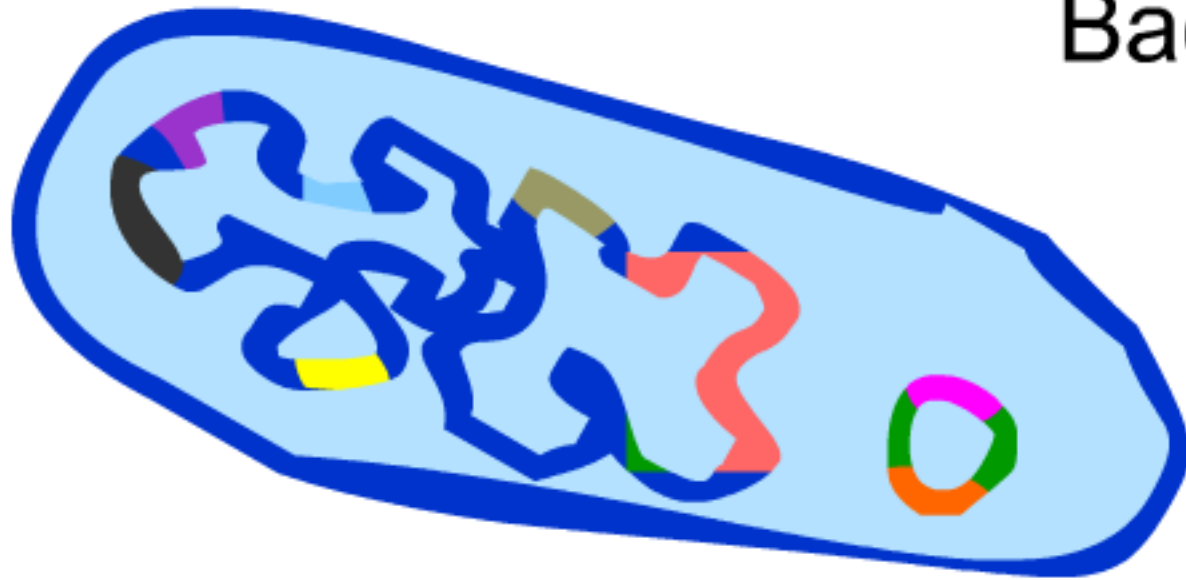
Antibiotic Resistance

Hendrickson



How is resistance evolving?

Bacteria are genetic chimeras.



Antibiotic Resistance

Hendrickson



How Bacteria Evolve

1) multiplication

2) heredity

3) variation

4) horizontal gene transfer

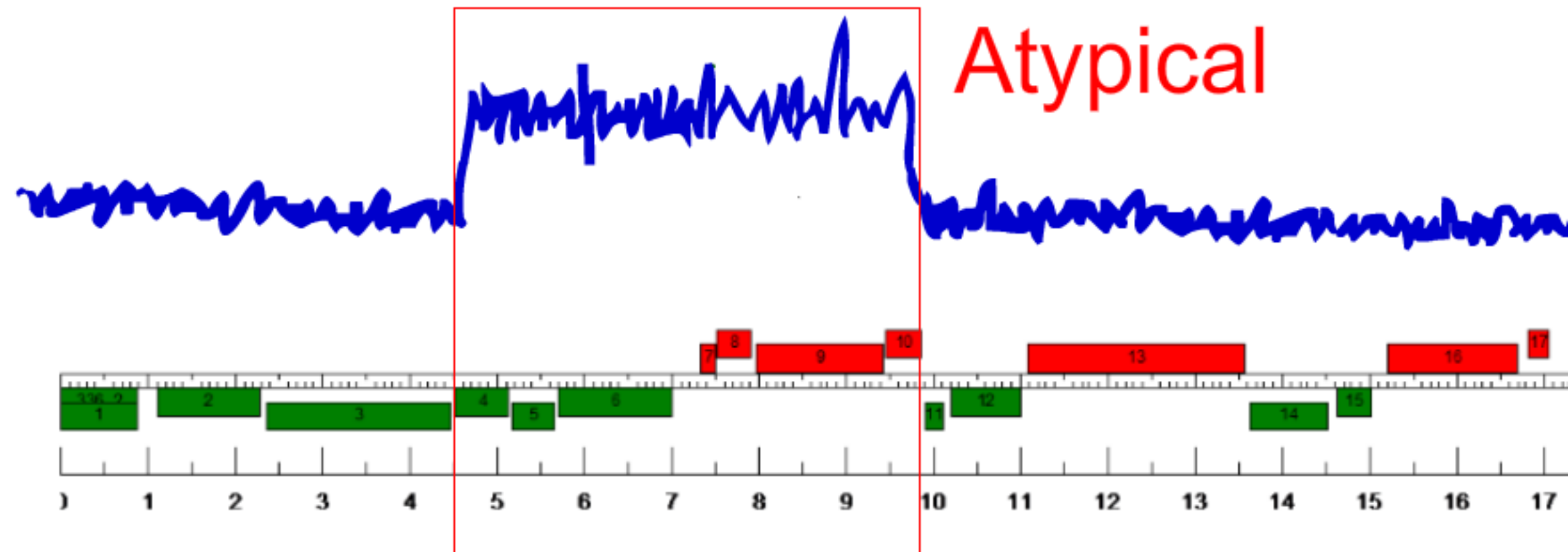


Evidence of genetic chimerism

Genome sequences:

GC
content

genes



prev

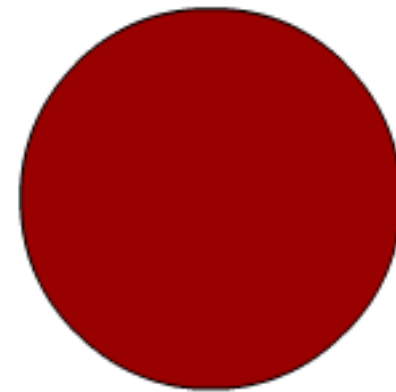
Antibiotic Resistance

Hendrickson



next

60 *E. coli* strains and genetic chimerism



Shared *E. coli* Genome
2,200 genes (98% similar)



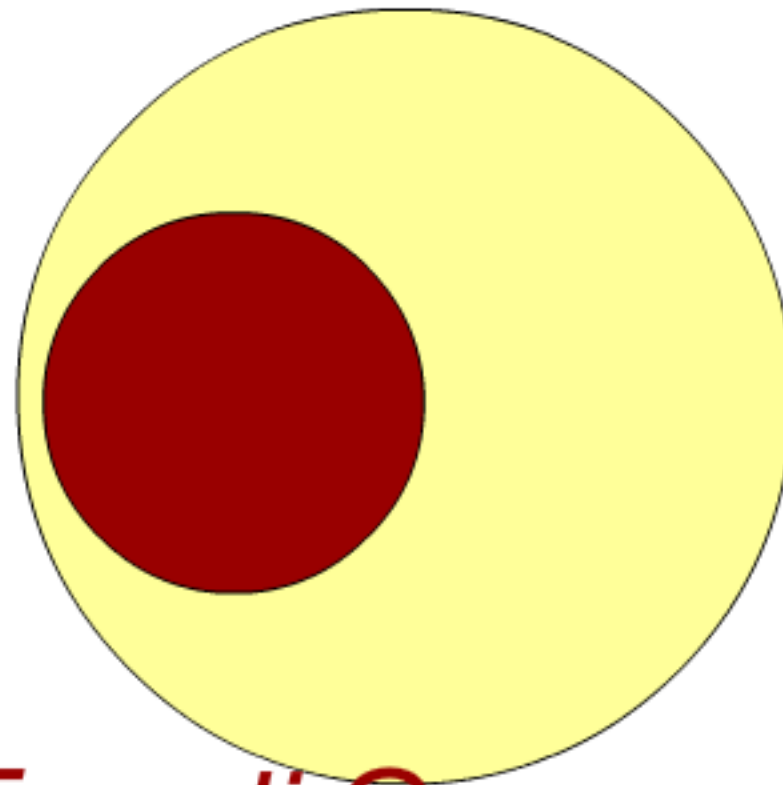
Antibiotic Resistance

Hendrickson



60 *E. coli* strains and genetic chimerism

Average *E. coli* 4,700 genes



Shared *E. coli* Genome
2,200 genes (98% similar)



60 *E. coli* strains and genetic chimerism

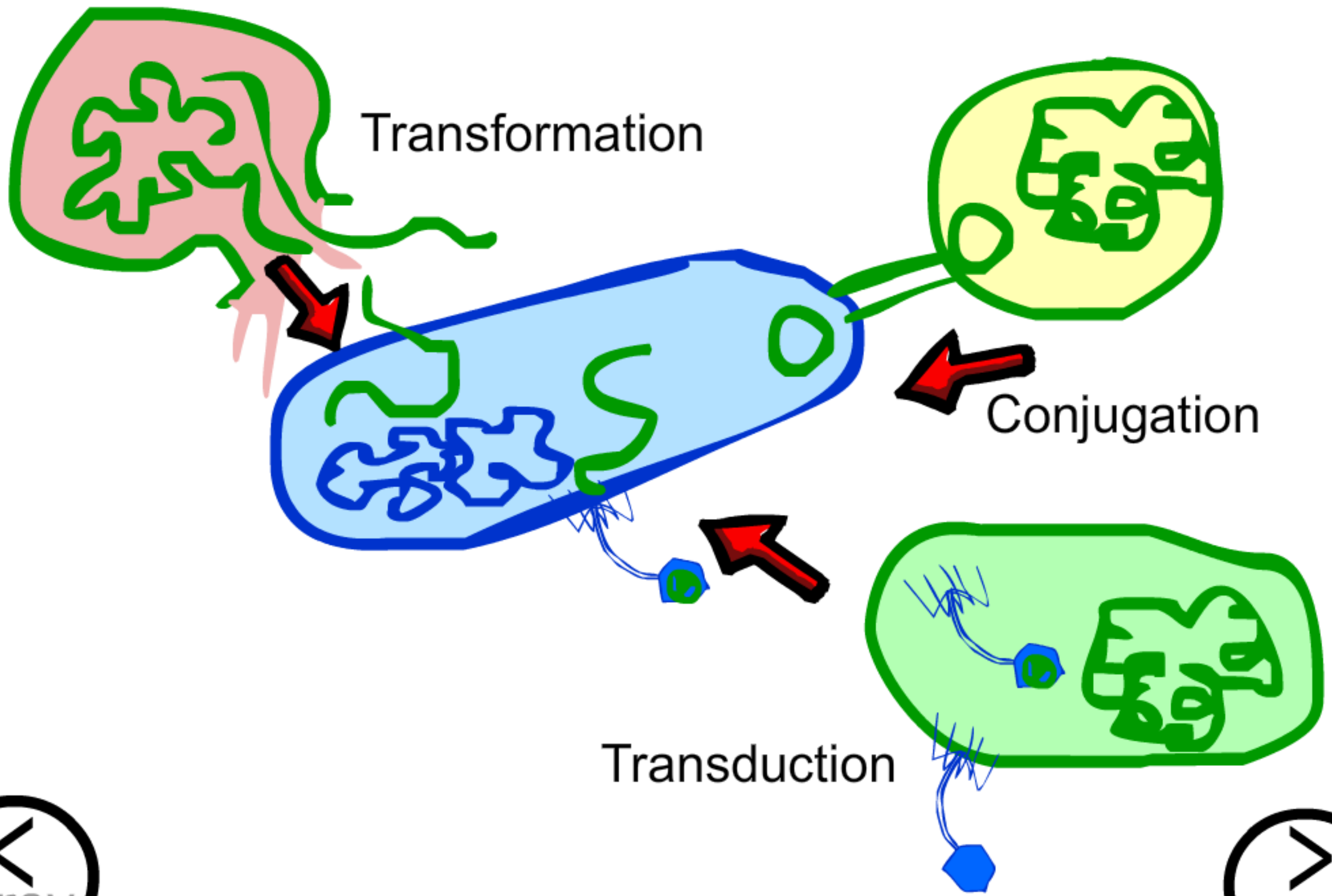
All potential *E. coli* genes: 10,100

Average *E. coli* 4,700 genes

Shared *E. coli* Genome
2,200 genes (98% similar)



Horizontal Gene Transfer

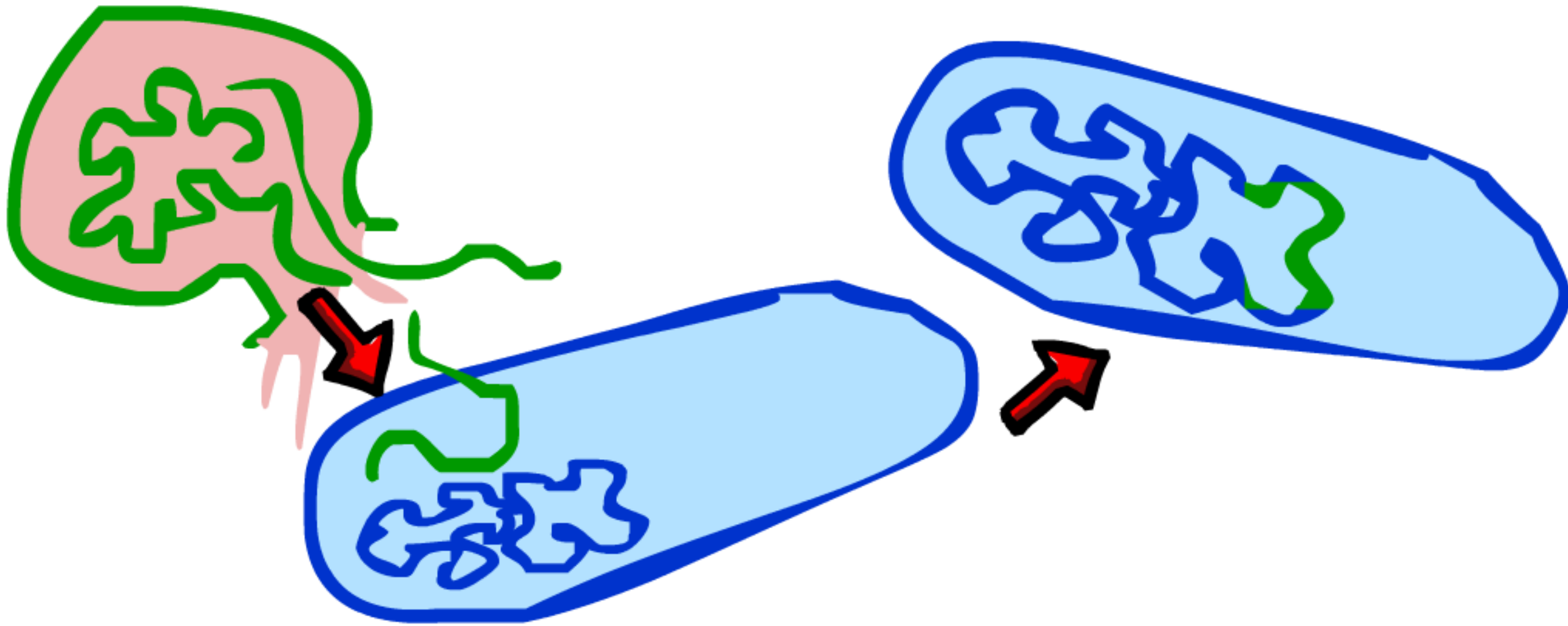


Antibiotic Resistance

Hendrickson



Natural Transformation



Naked DNA in the environment is 'delicious'.

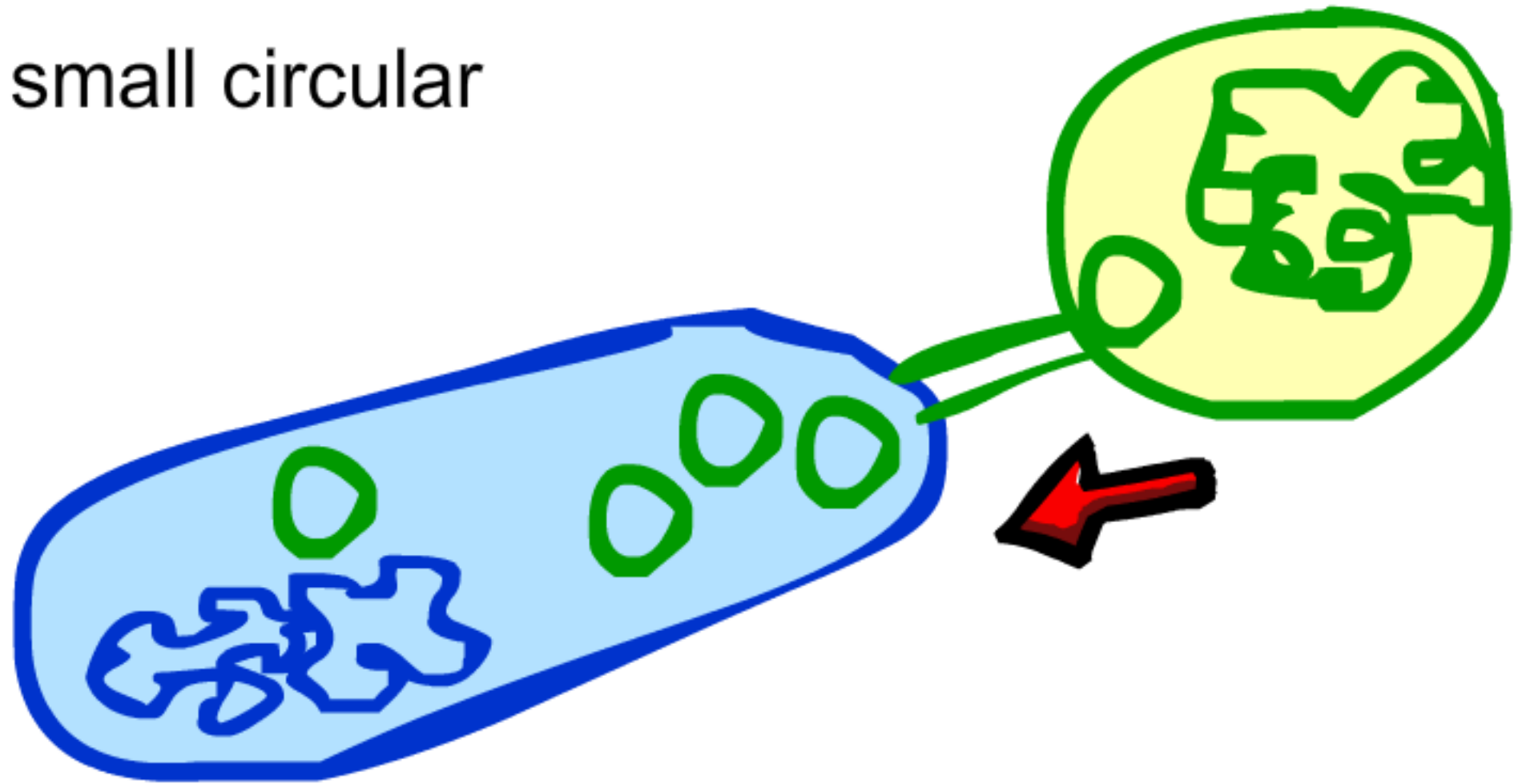
Some bacteria take this up as food.

Novel DNA can recombine into the bacterial DNA



Conjugation

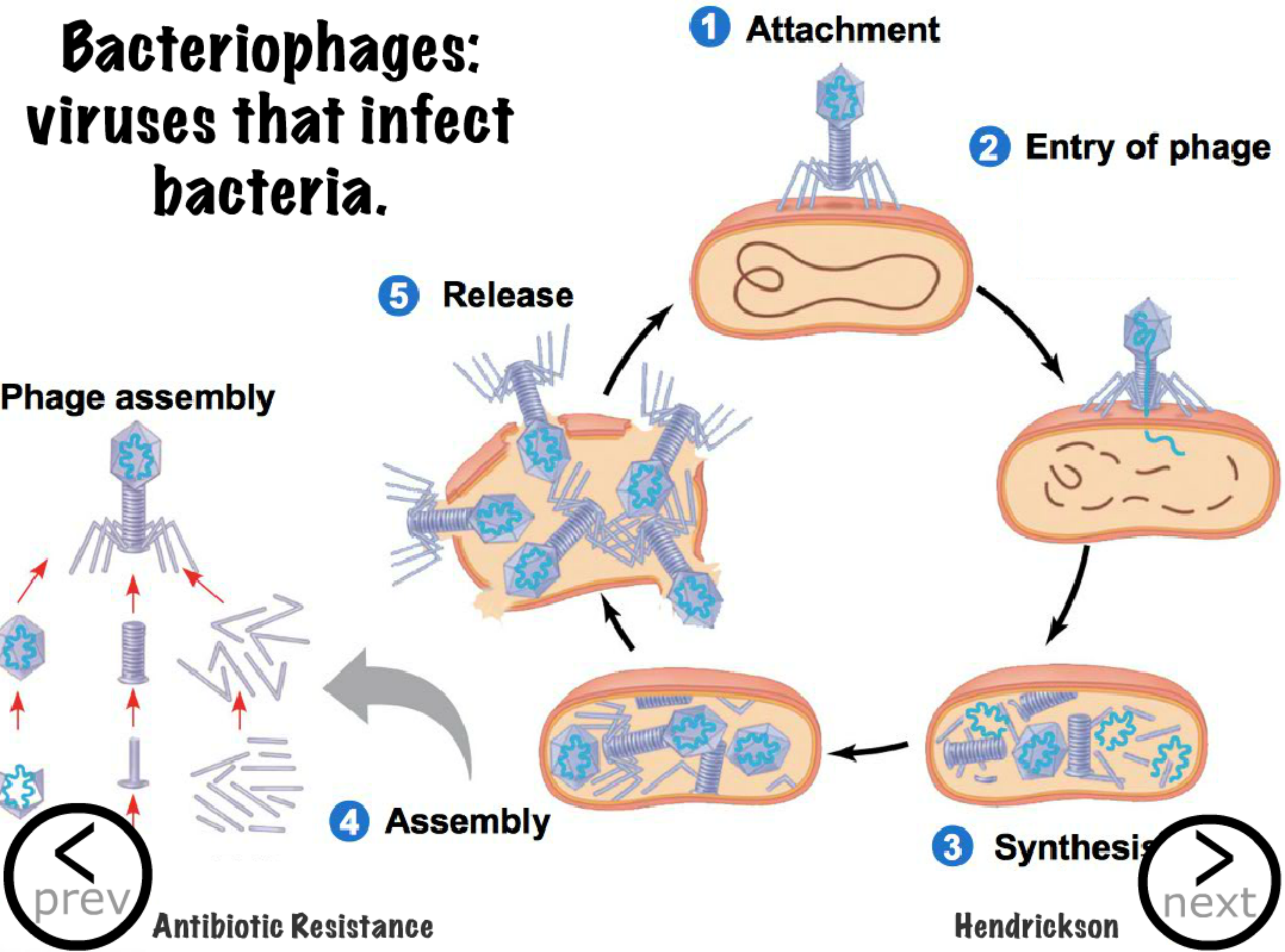
Plasmids are small circular DNA loops.



Replicate themselves independently in cells.

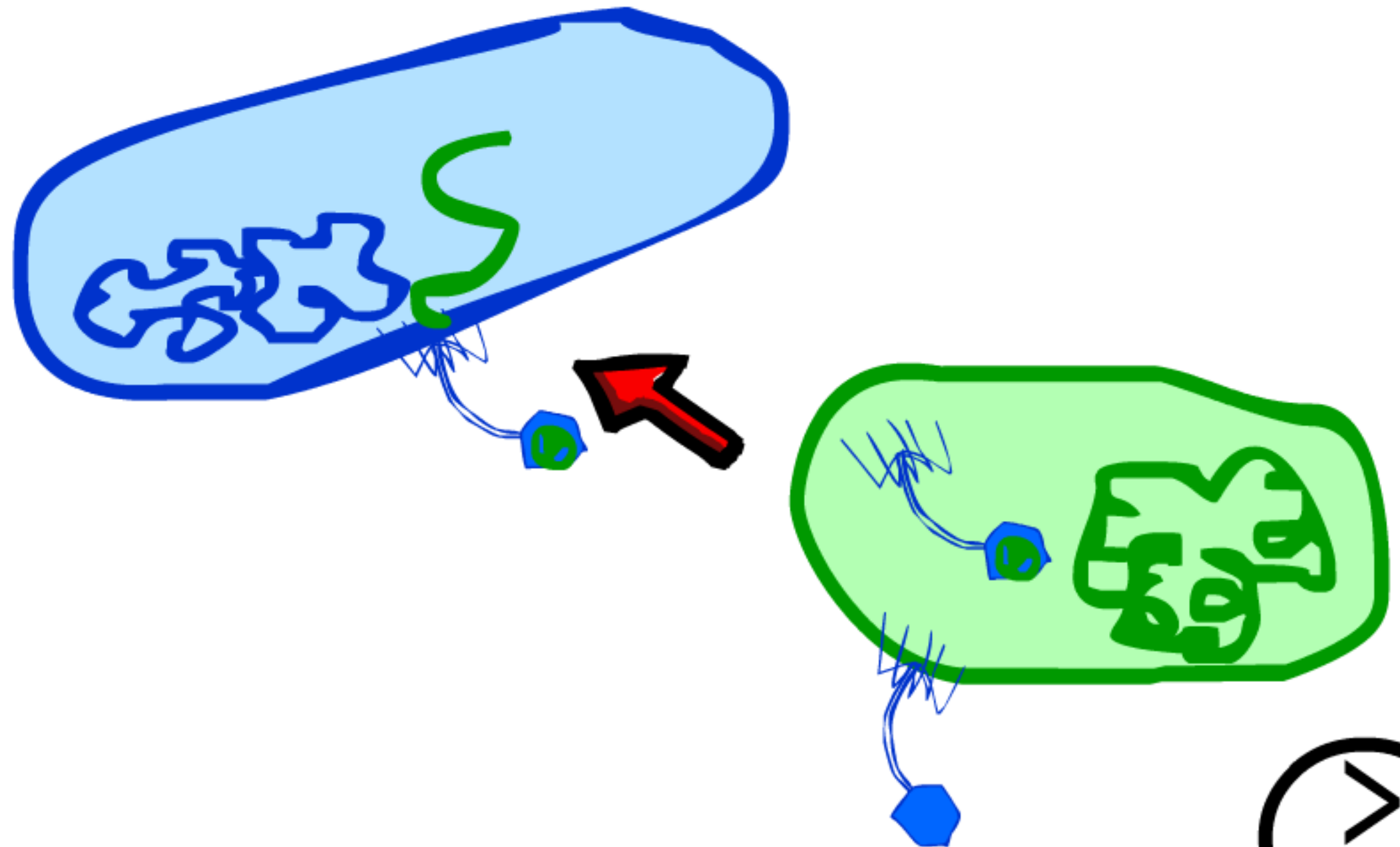
Plasmids often transfer between strains or species.

Bacteriophages: viruses that infect bacteria.



Transduction

Bacteriophage mistakenly packages bacterial DNA.
Next infection this foreign DNA is injected.
Foreign DNA can recombine into bacterial DNA.

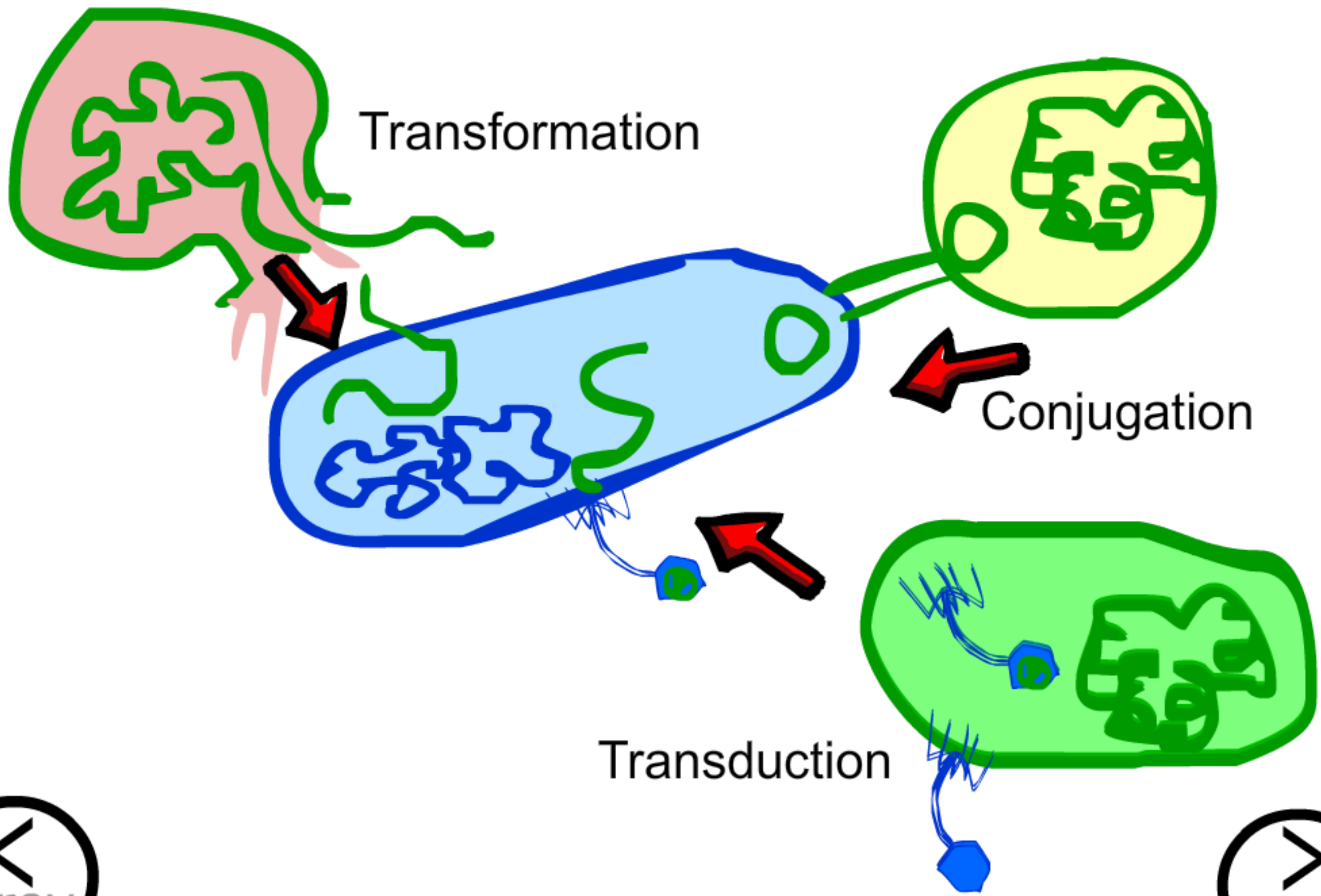


Antibiotic Resistance

Hendrickson



HGT takes place in a single cell



Antibiotic Resistance

Hendrickson



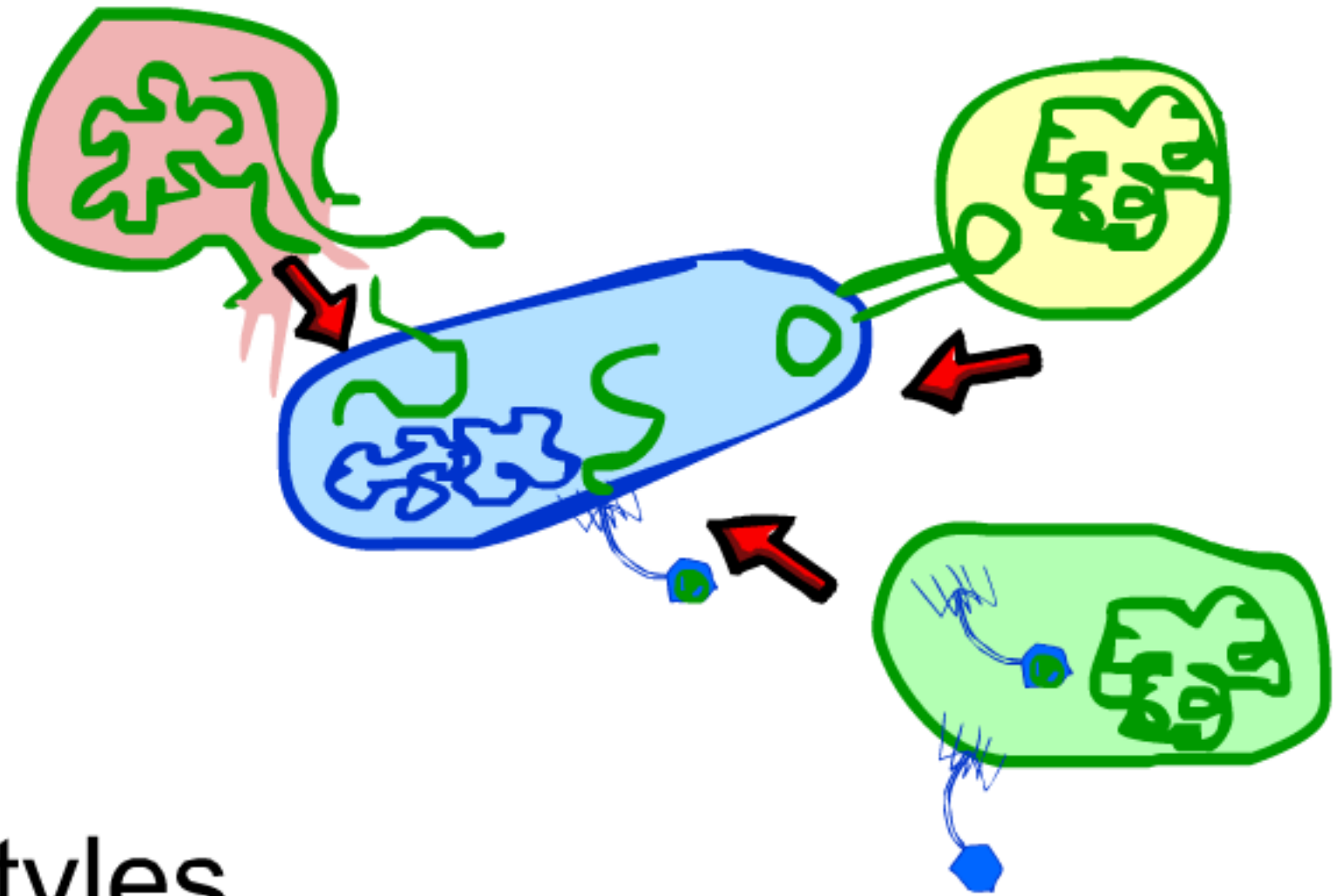
Selection for HGT

Any & All Genes:
toxicity, metabolic,
antibiotic resistance.

New combinations
mean new novel lifestyles.

Fast forward evolution.

Remember HGT
is accidental...



Antibiotic Resistance

Hendrickson

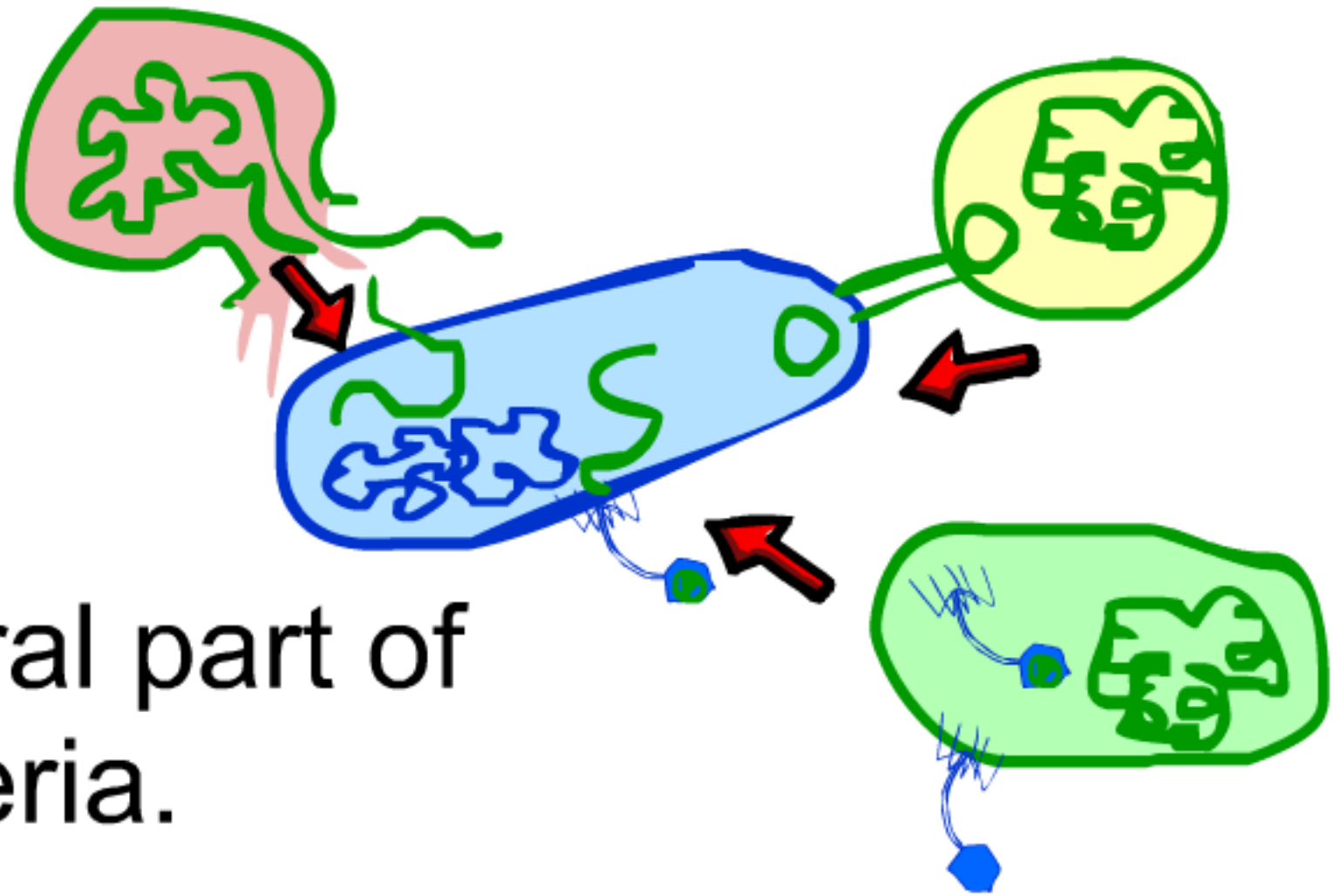


There is no stopping HGT

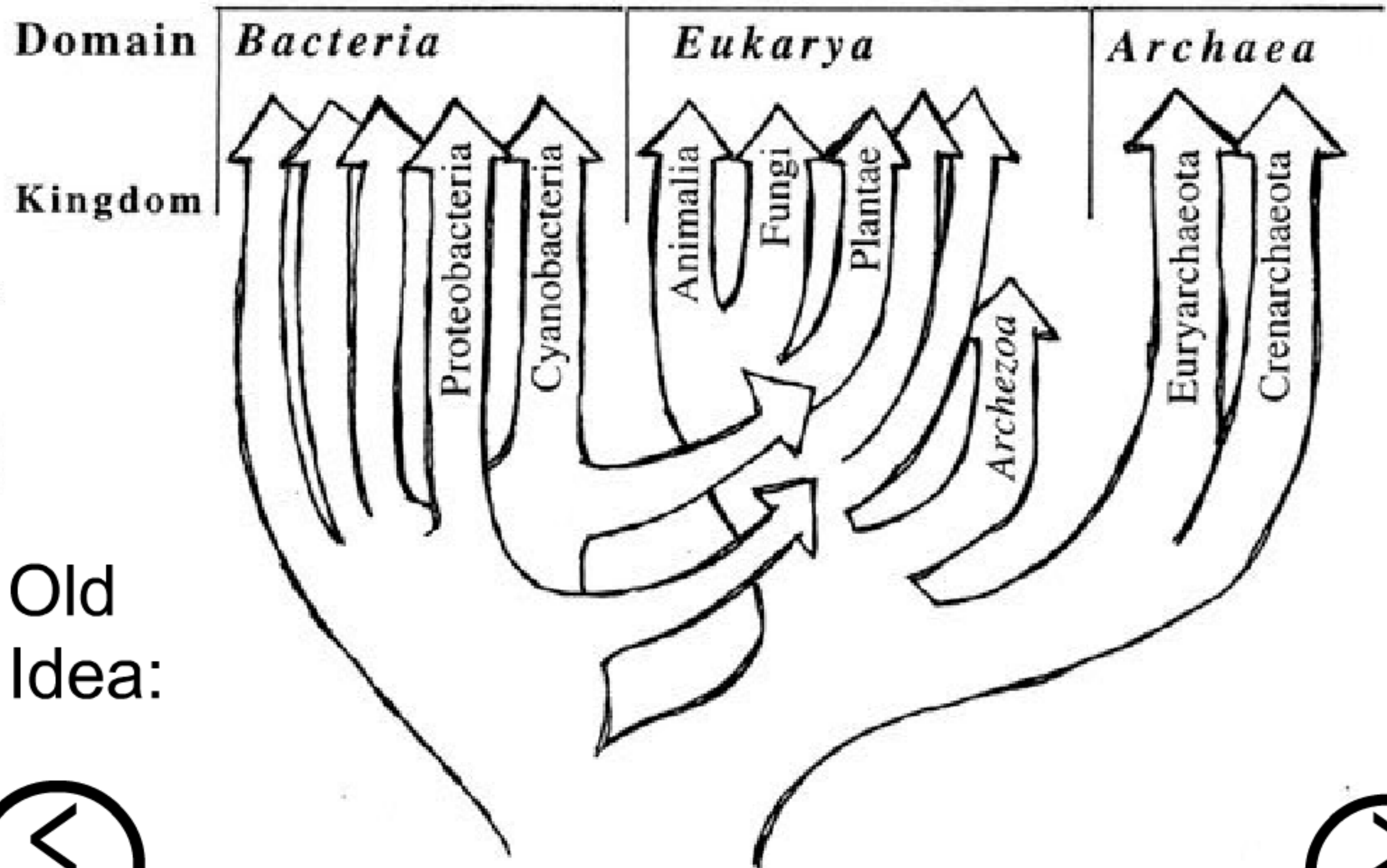
Plasmids:
constantly evolving &
numerous.

Transformation: natural part of
eating for some bacteria.

Bacteriophages: 10 times more
numerous than bacteria.



Consequences of HGT



Old
Idea:

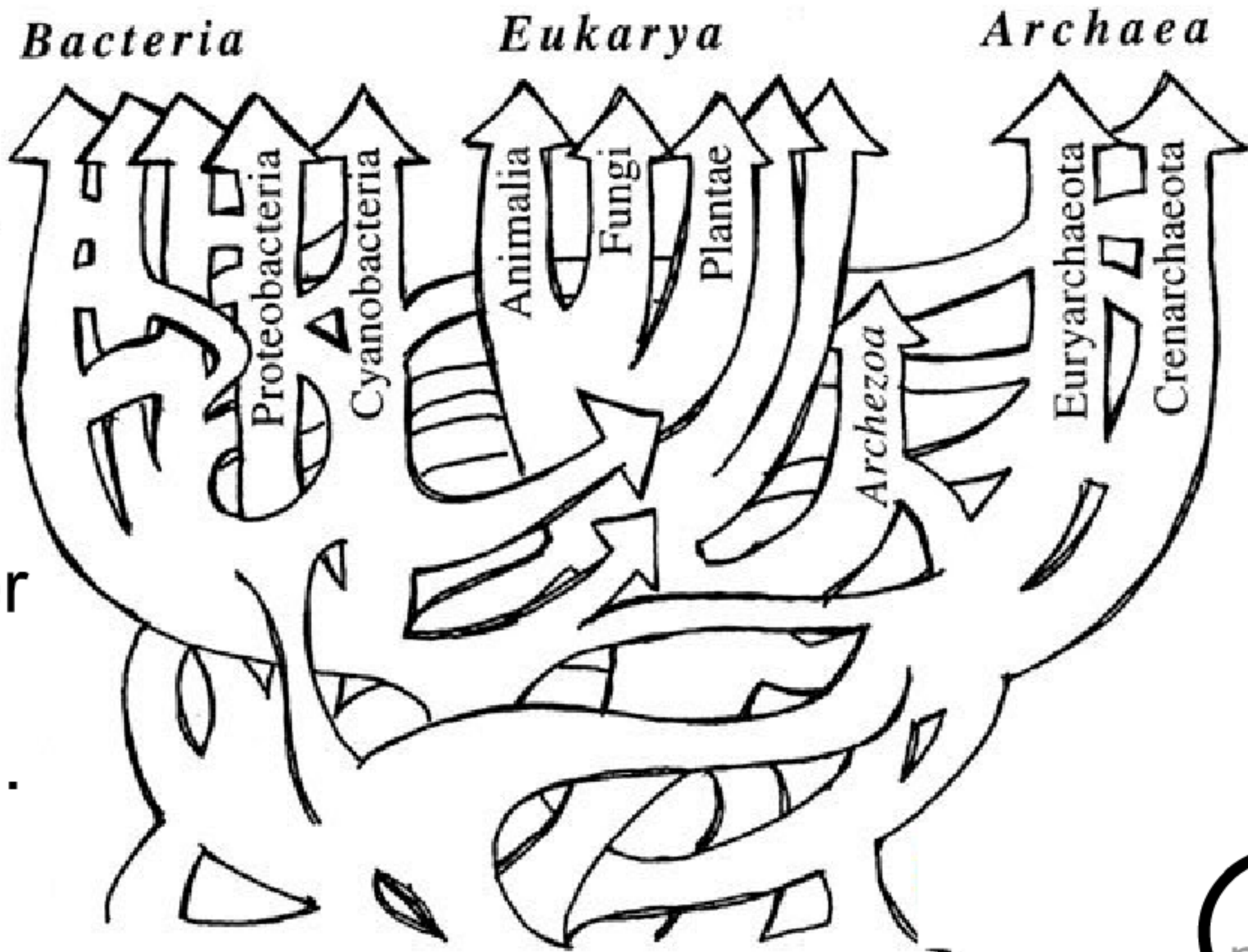


Antibiotic Resistance

Hendrickson



Consequences of HGT



Closer
to the
truth...



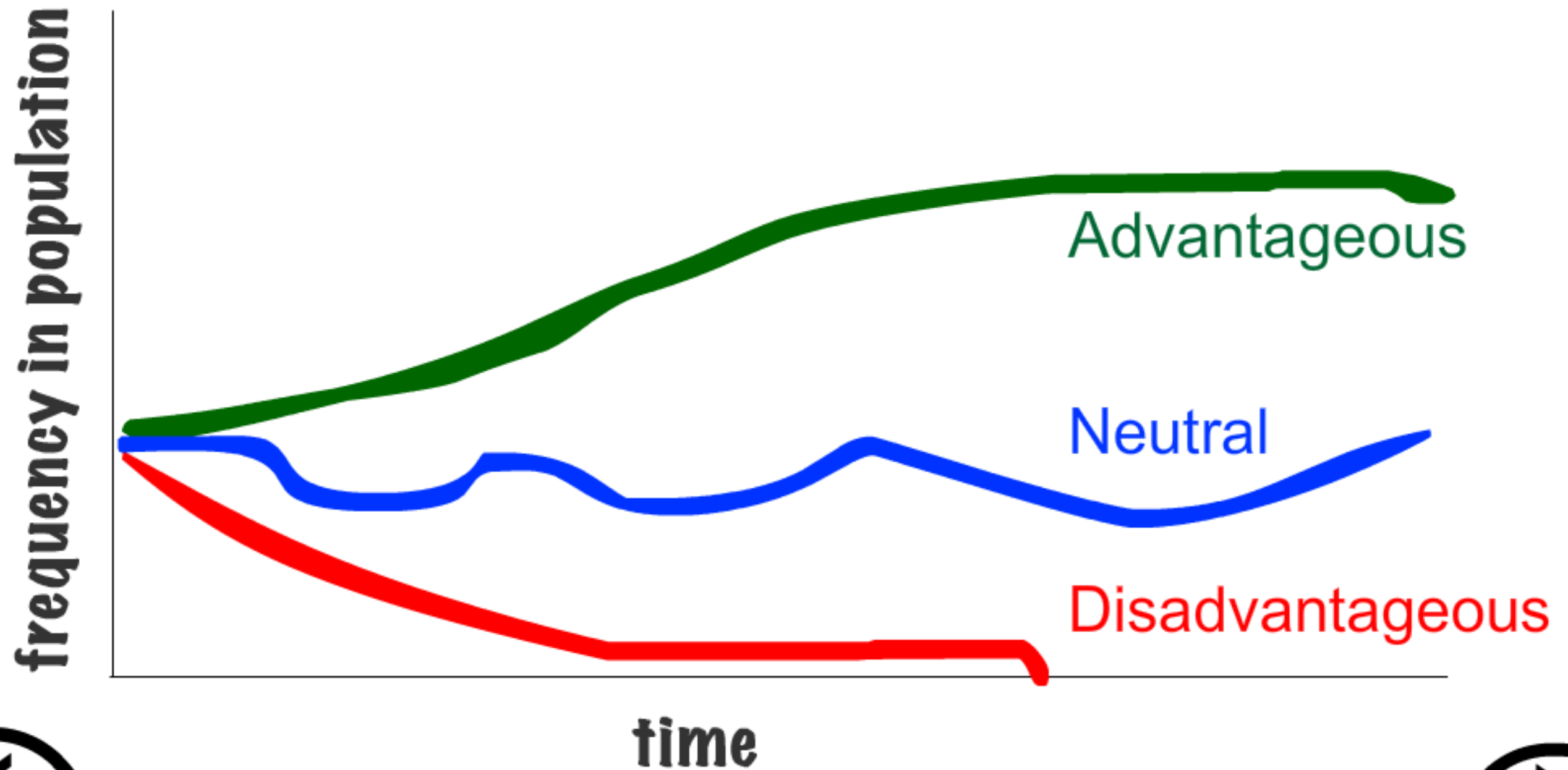
Antibiotic Resistance

Hendrickson



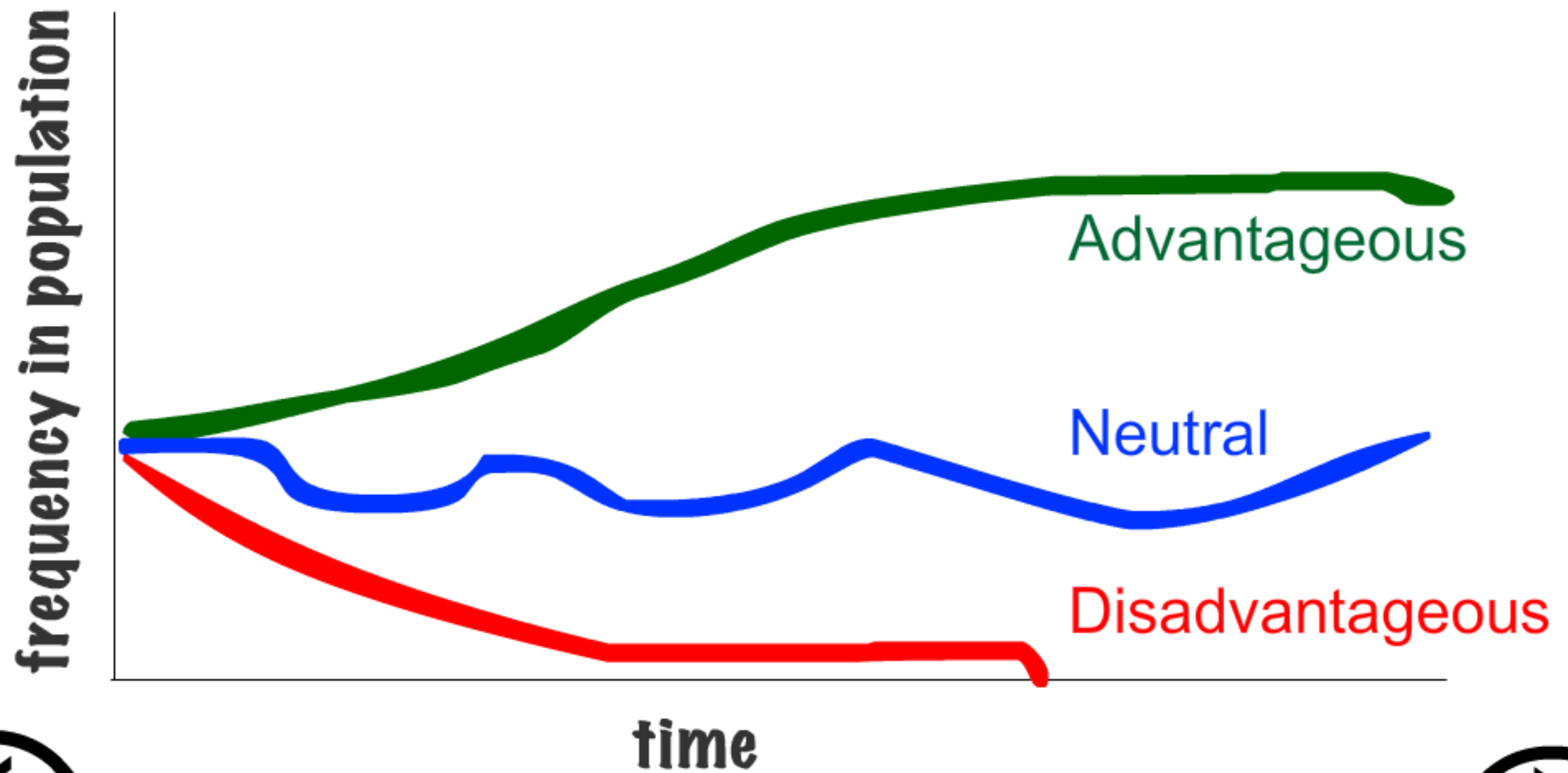
Consequences of HGT in population depends on the environment

This will effect how the cell fares competitively:



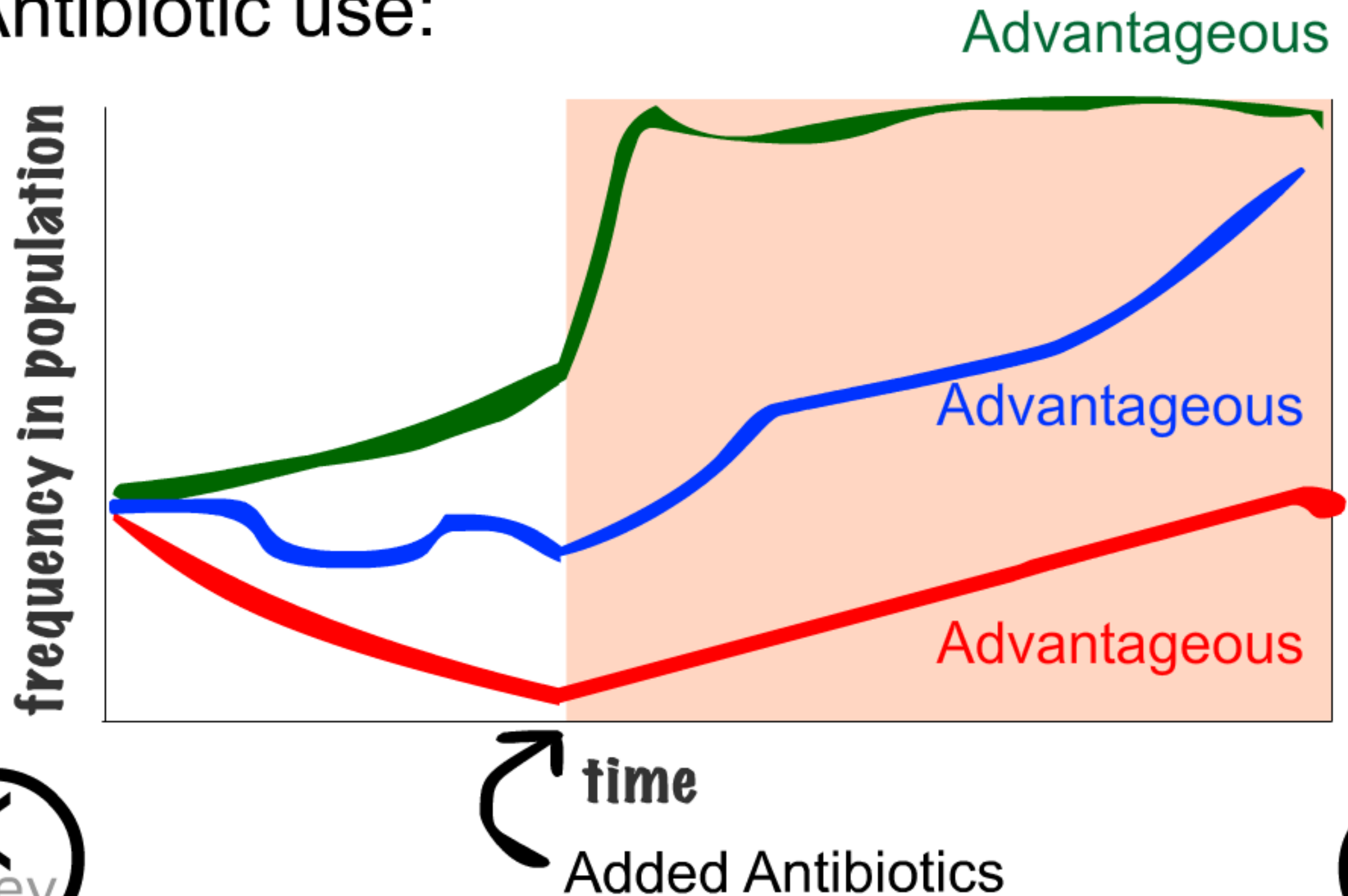
How are we effecting HGT?

Antibiotic use:



We Provide the Selective Pressure to Increase Fitness/ Frequency

Antibiotic use:



Antibiotic Resistance

Hendrickson

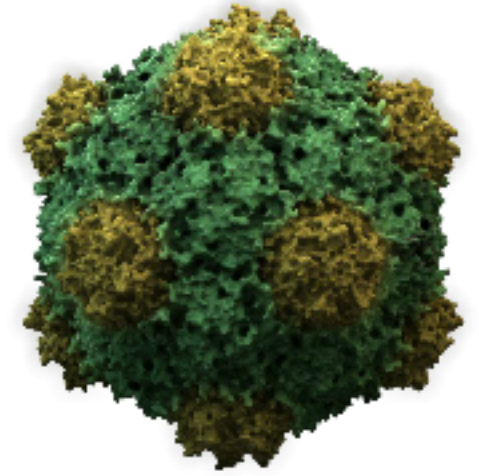


Where is HGT taking place?

Where is HGT **not** taking place?



How are we effecting HGT?



Antibiotic use:

Over-prescription:
55% of ABs for
respiratory tract
infections
unnecessary

Overview of Viral infections

Encephalitis/
meningitis

Common cold

Eye infections

Gingivostomatitis

Parotitis

Pneumonia

Cardiovascular

Hepatitis

Myelitis

Skin infections

Gastroenteritis
allus

Sexually transmitted
diseases



Antibiotic Resistance

Hendrickson



How are we effecting HGT?

Antibiotic use:

Uneccessary AB use on farms

-antimicrobials are used in healthy animals to encourage growth.



Antibiotic Resistance

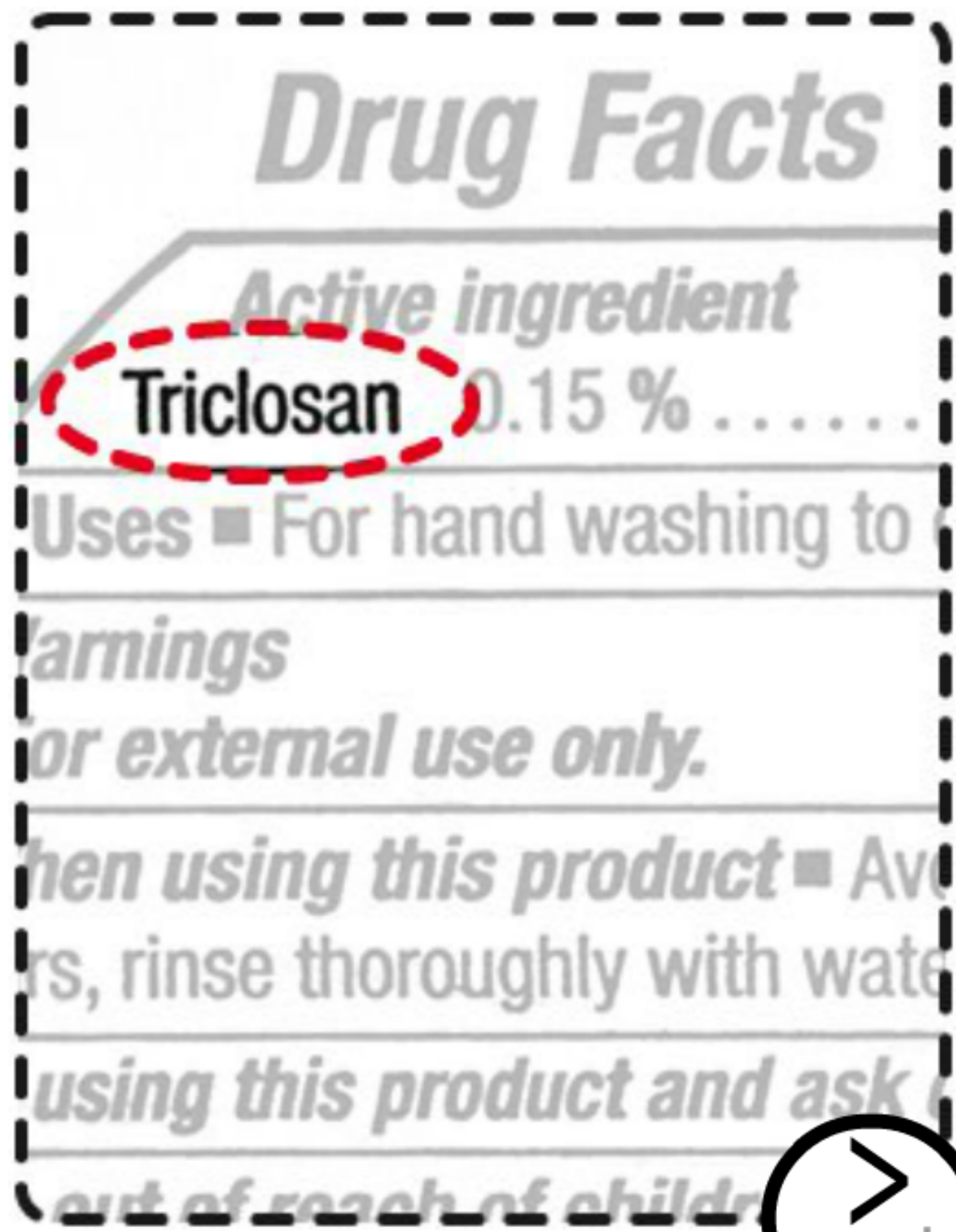
Hendrickson



How are we effecting HGT?

Antibiotic use:

Antimicrobial soaps
-triclosan, inhibits an enzyme so low levels select for resistance.

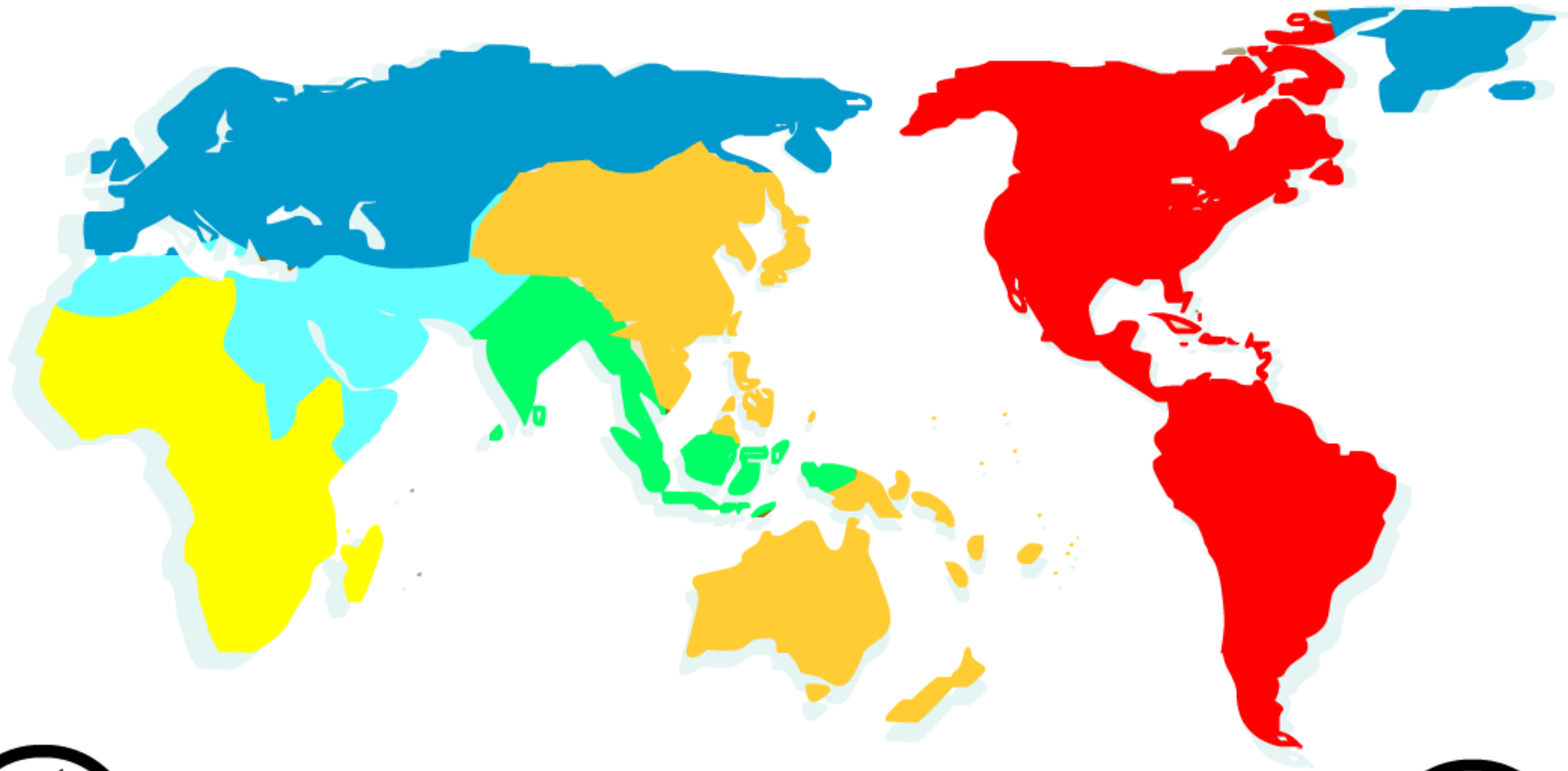


Antibiotic Resistance

Hendrickson



How bad is antimicrobial resistance now?



Antibiotic Resistance

WHO Report (2014)

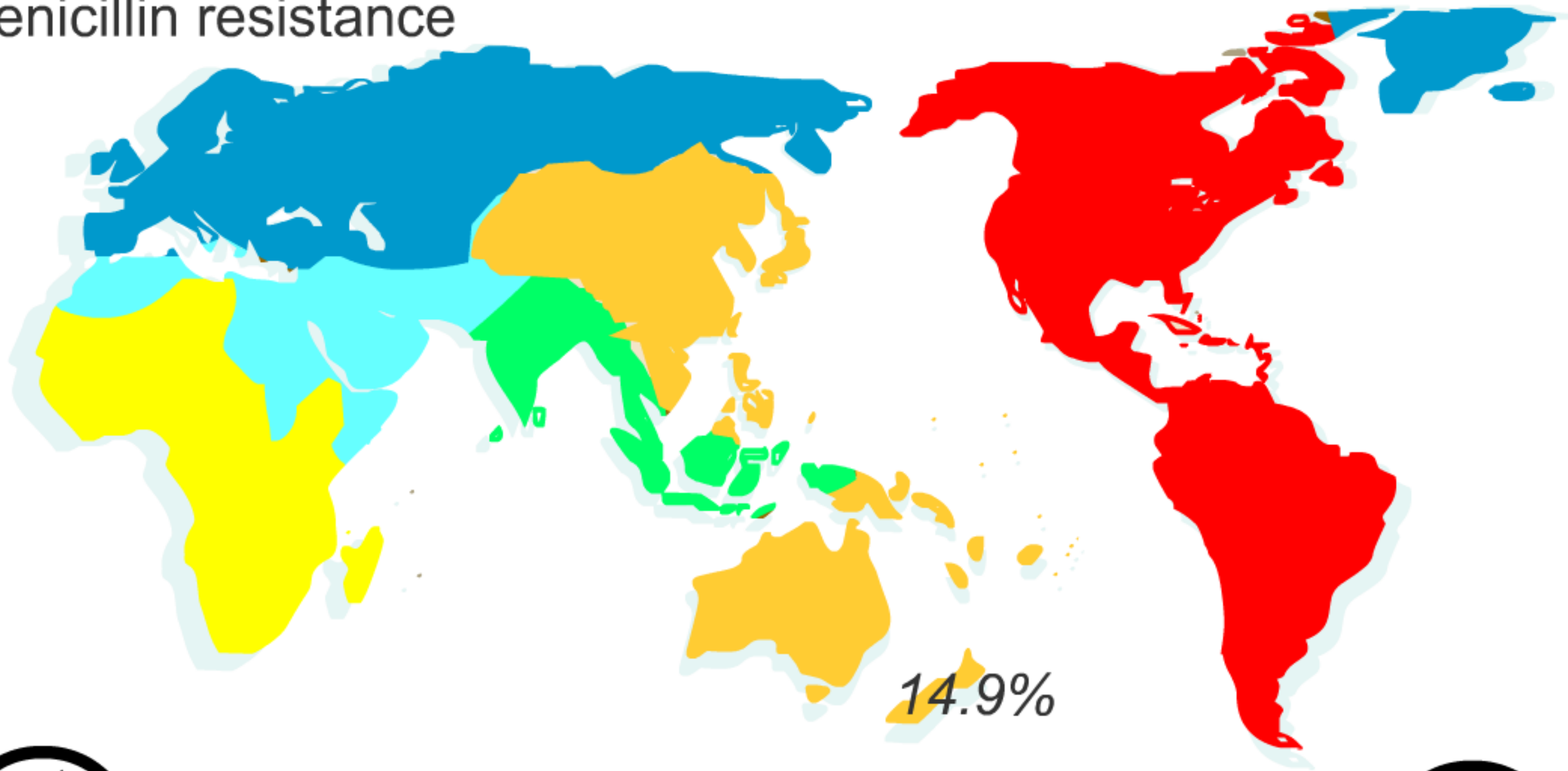
Hendrickson



How bad is antimicrobial resistance now?

Streptococcus pneumoniae:

Penicillin resistance



Antibiotic Resistance

WHO Report (2014)

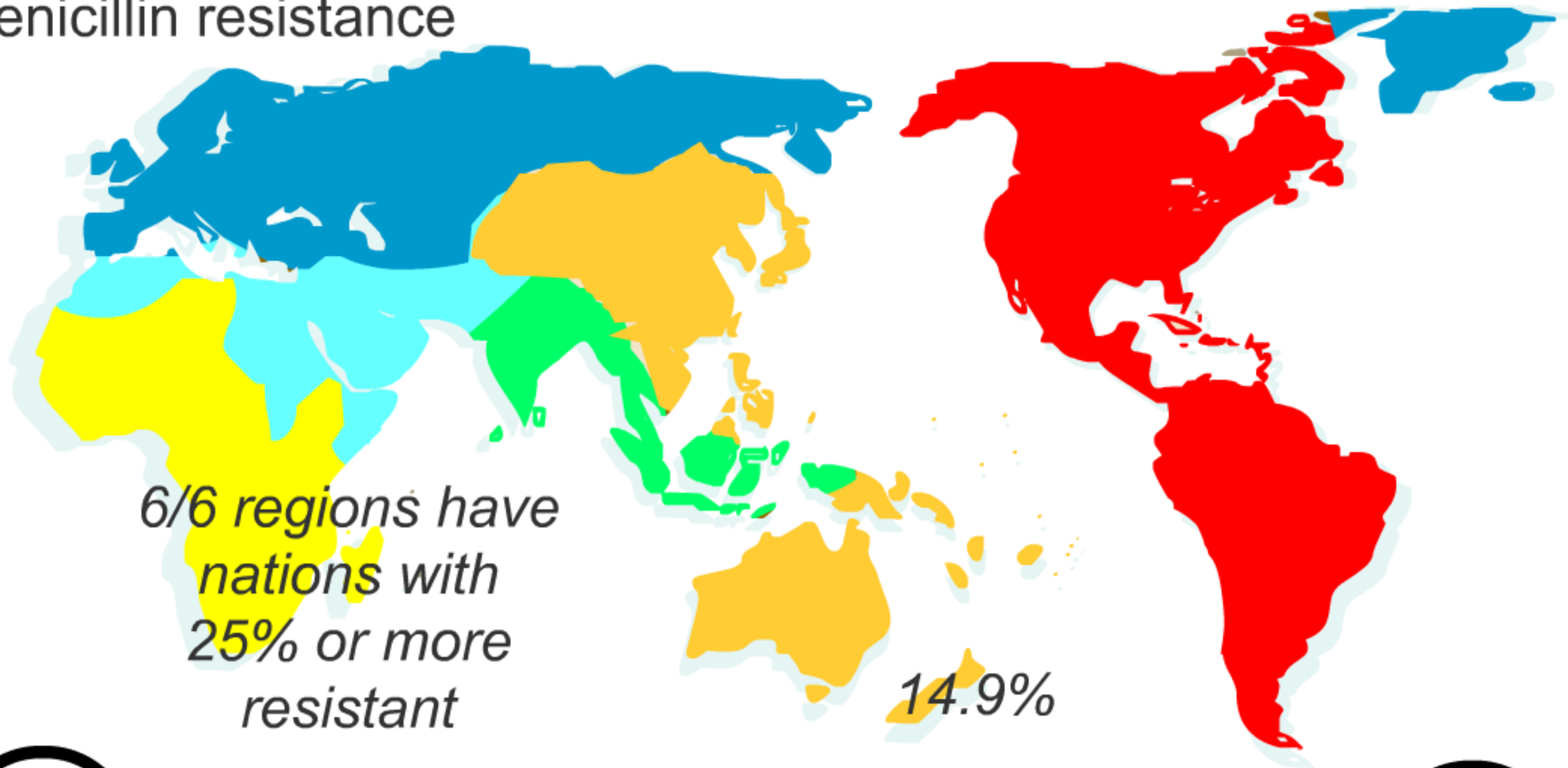
Hendrickson



How bad is antimicrobial resistance now?

Streptococcus pneumoniae:

Penicillin resistance



6/6 regions have
nations with
25% or more
resistant

14.9%

WHO Report (2014)

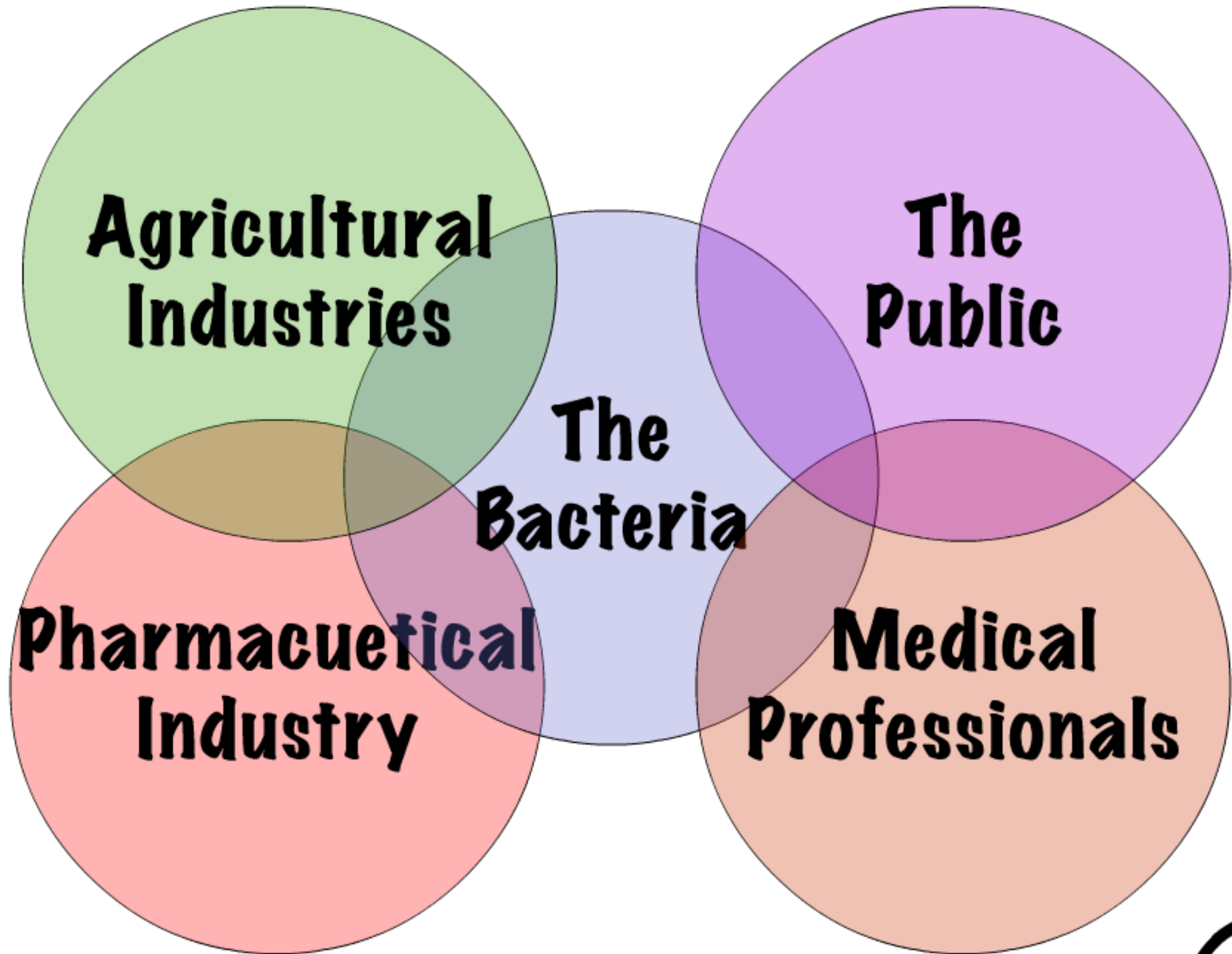


Antibiotic Resistance

Hendrickson



The Players in Antibiotic Resistance



Questions:

Bacteria, a microbiologists perspective.

What are antimicrobials ?

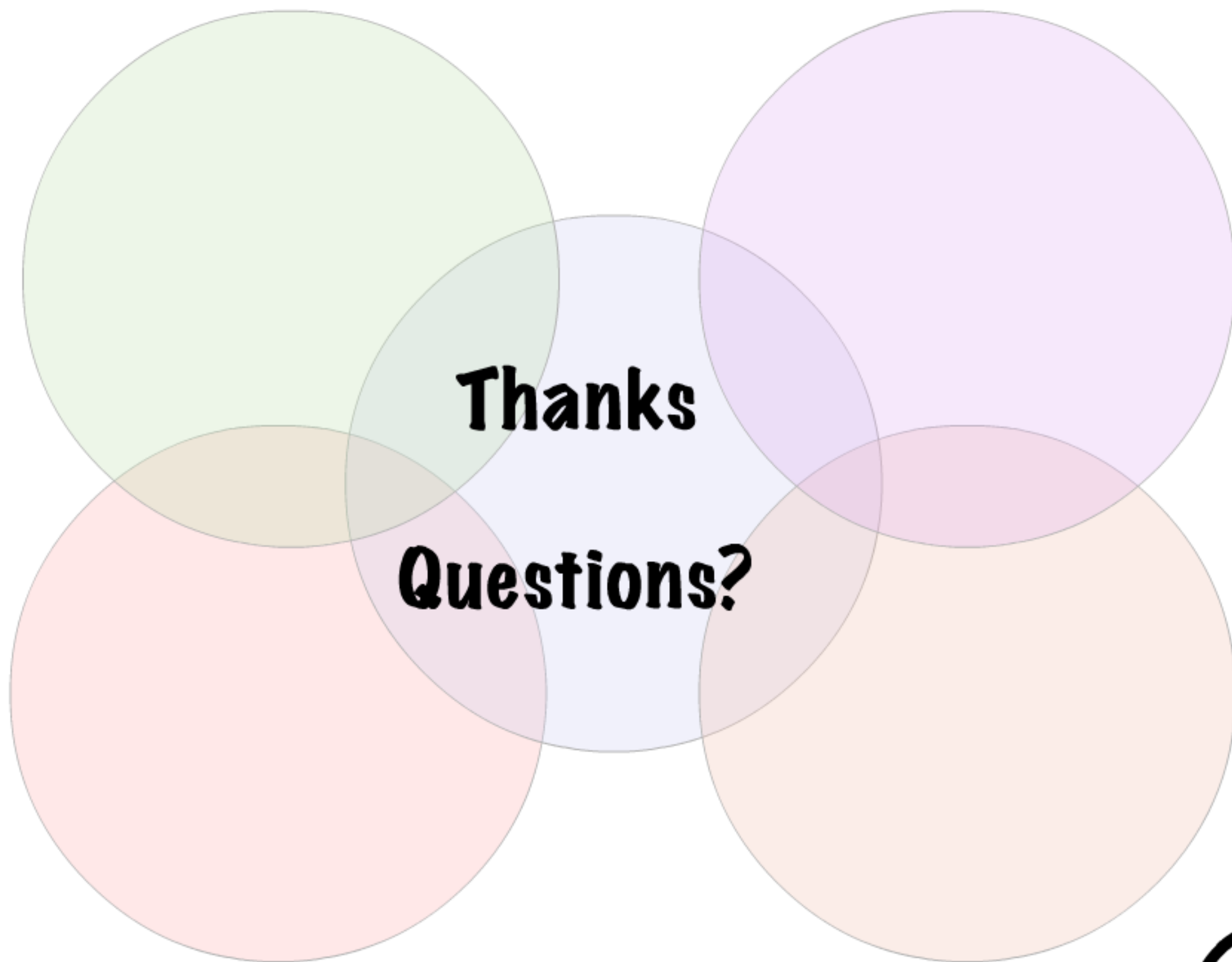
Where does antimicrobial resistance come from?

Do antimicrobial products matter?

How bad is the problem is it?

What can we do?

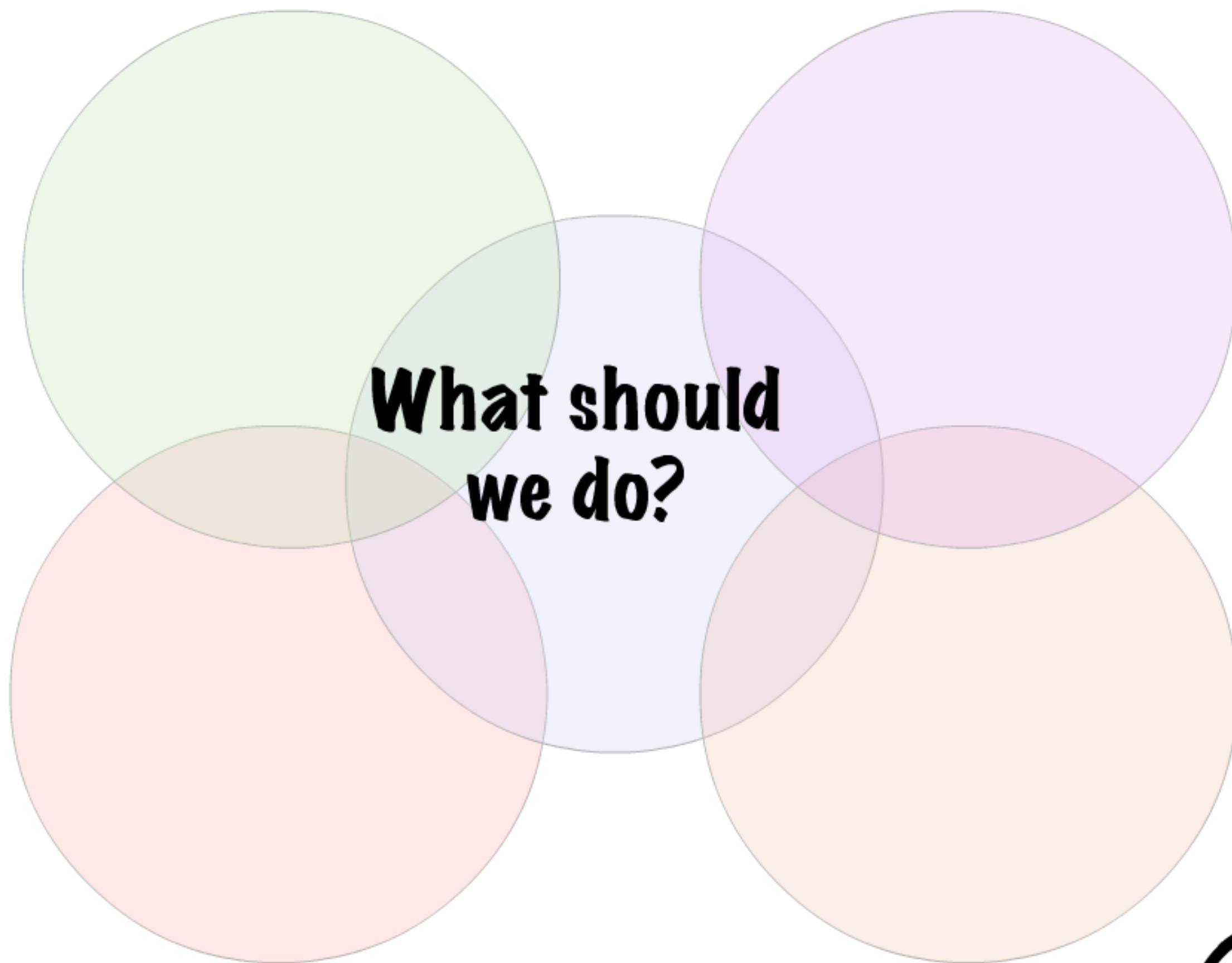




Antibiotic Resistance

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Antibiotic Resistance

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Cut back on antibiotic use

Shifting recommendations:

Broad -> Narrow spectrum ABs

Finish AB course

Shorter courses of ABs

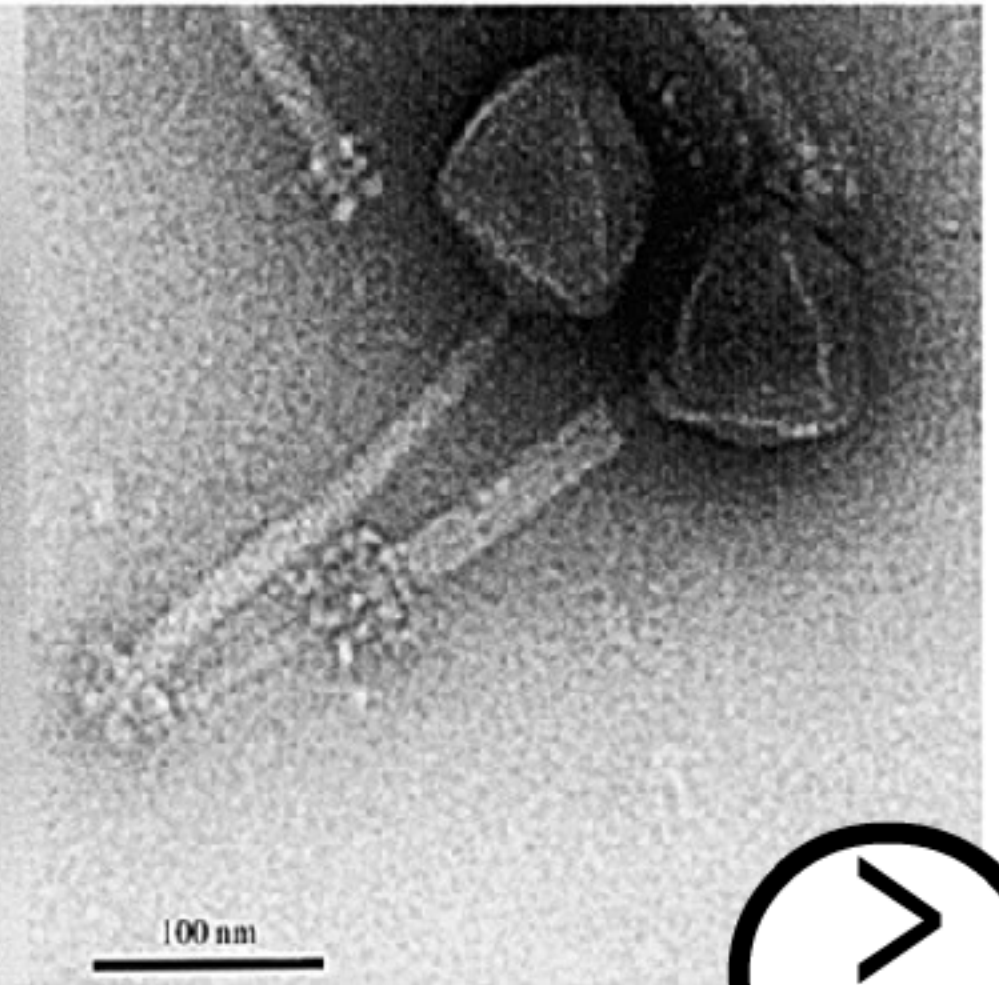
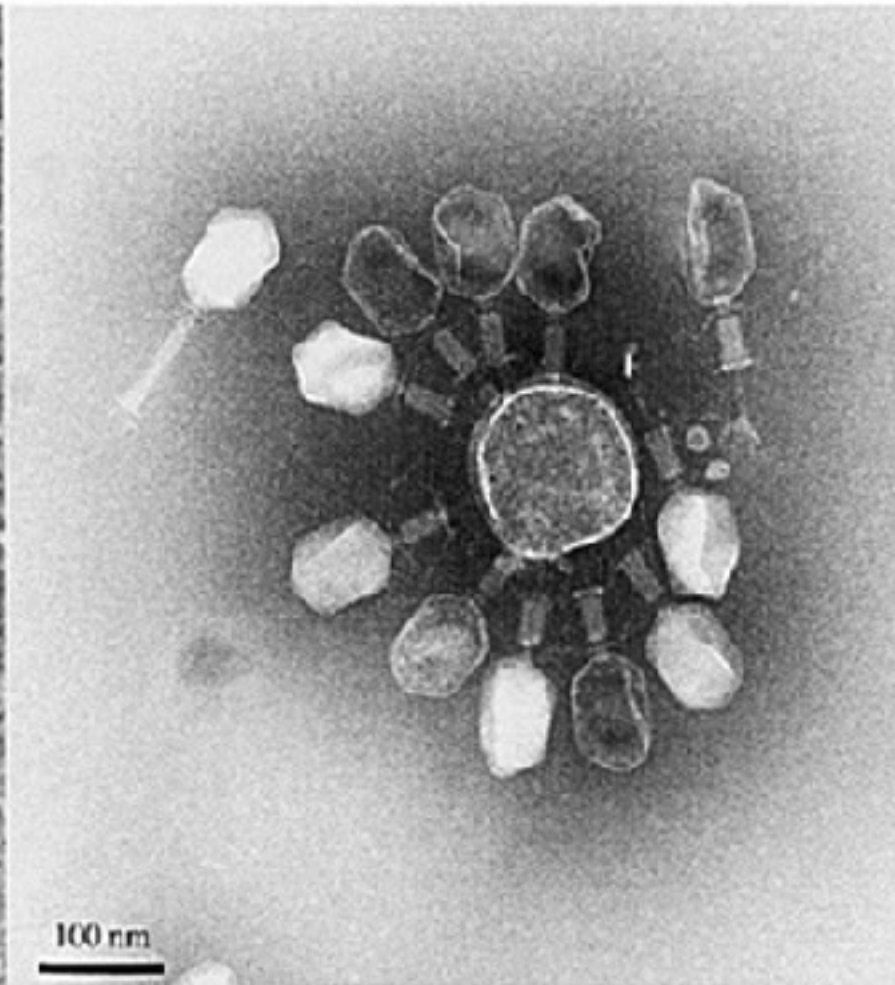
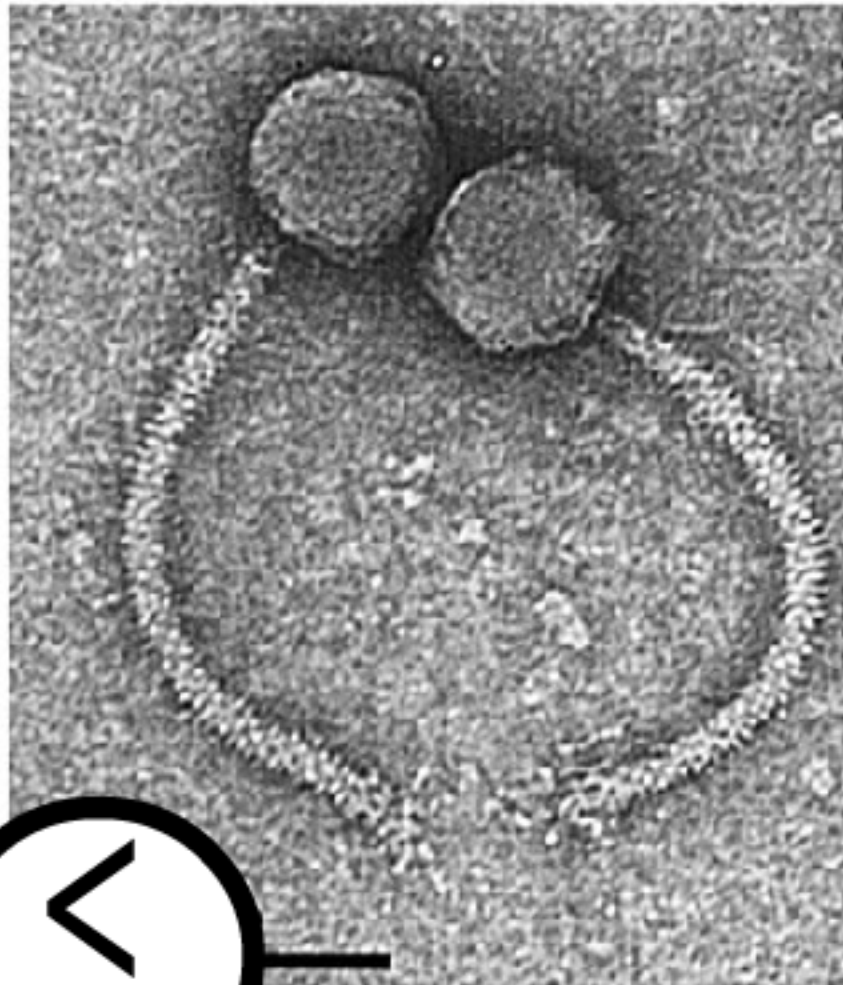


Phage Therapy

Therapeutic use of phage to treat bacterial infections

Phage are more specific than antibiotics

Can be chosen to cause no harm to animals and beneficial bacteria.



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Predatory Bacterial Therapy

***Bdellovibrio
bacteriovorus***



DARPA:

Toxicity to host?

Specificity?

Evolve resistance?



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Fecal Transplant Therapy:



Clostridium difficile

profuse diarrhea, uncontrollable vomiting and high fevers 14,000 deaths per year in the USA

Journal of New England Medical Study:



3/13

4/13

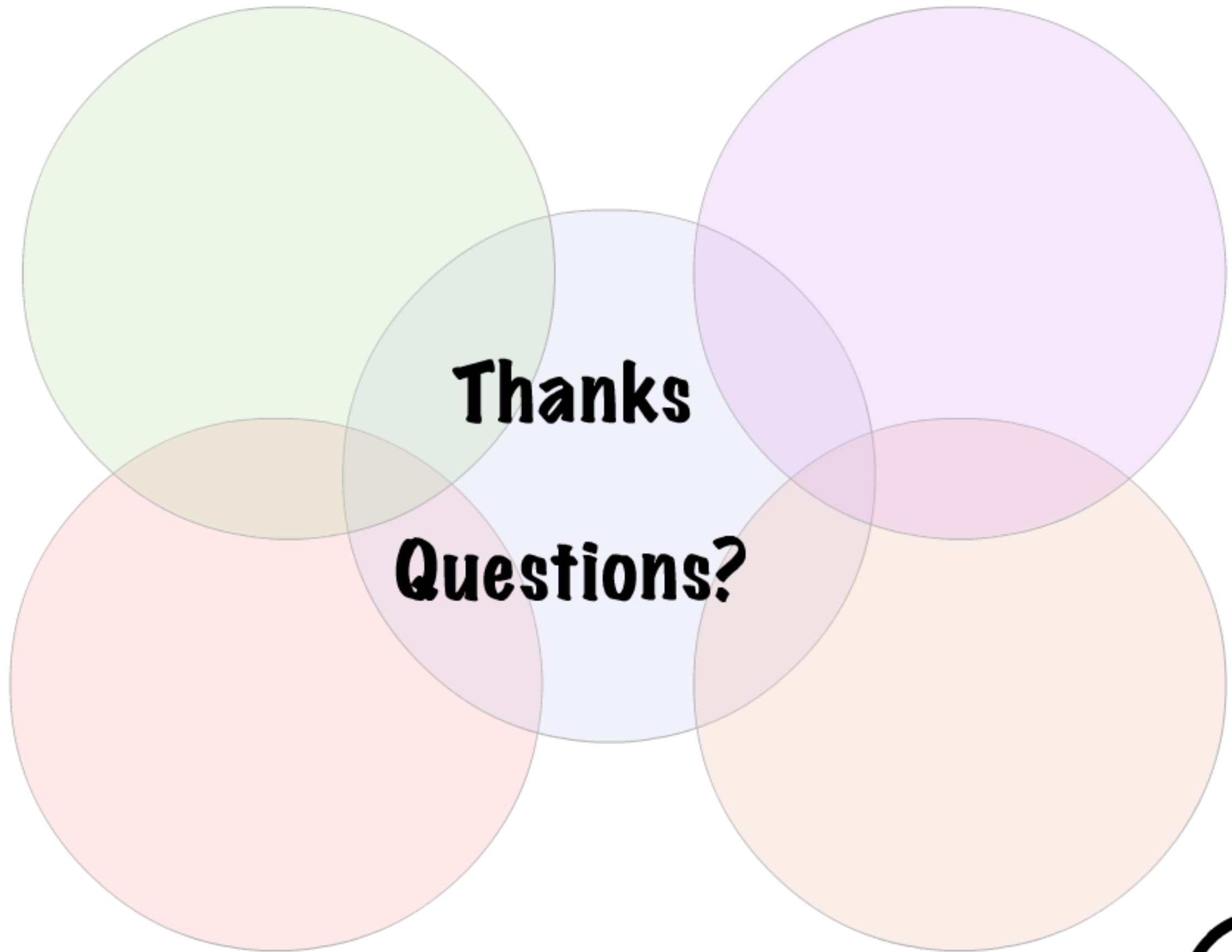
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HGT and Clostridium?



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Case Study: *Clostridium botulinum* *Clostridium sporogenes*

C. b. = one of the most important pathogens associated with food

C. s. = a harmless spore forming bacteria found in soil.

time 12 and 36 hrs after eating
contaminated food:

nausea,

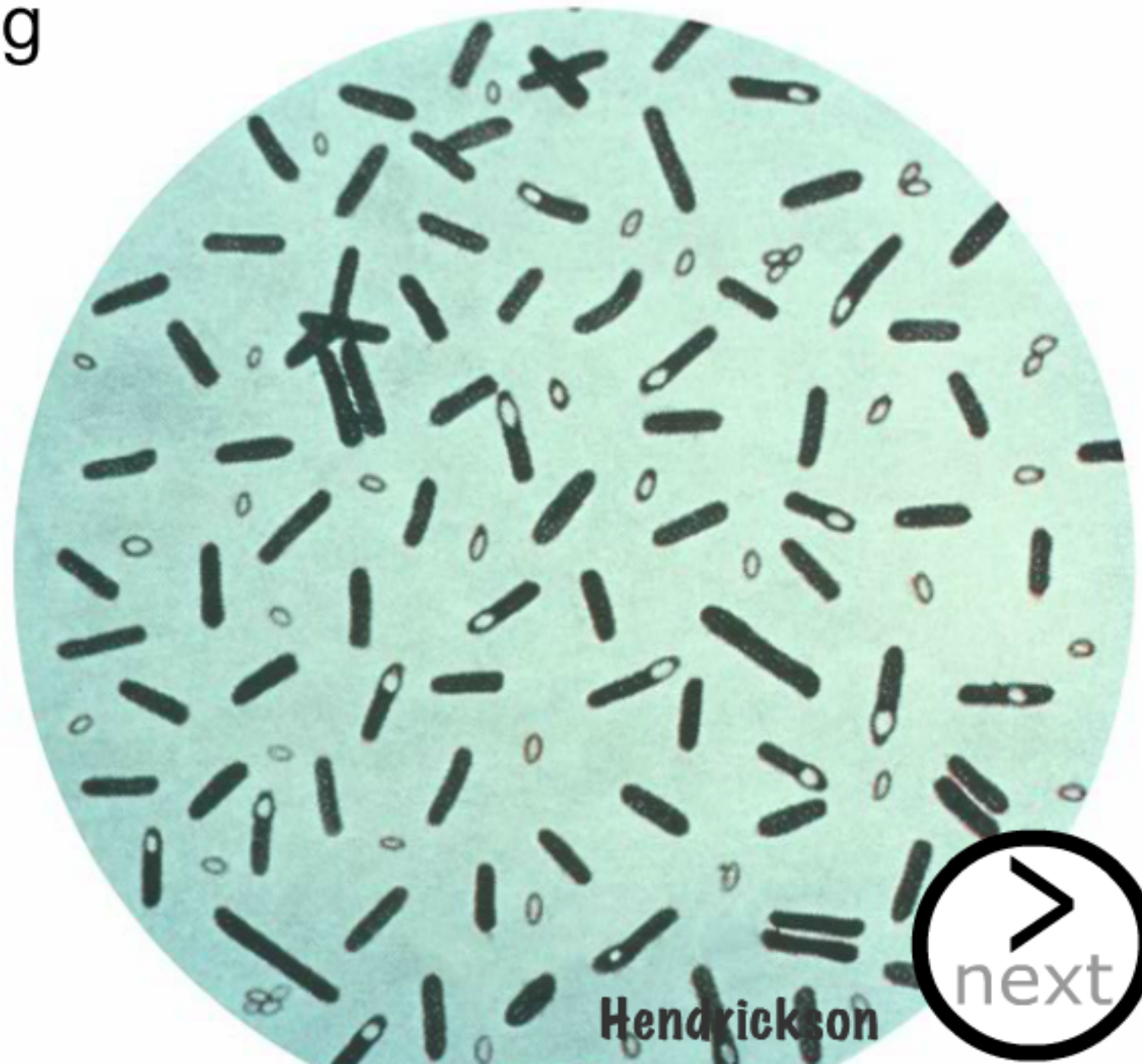
vomiting and

diarrhoea

paralysis of the

eyes, mouth, throat and,

progressively muscles



Antibiotic Resistance

time

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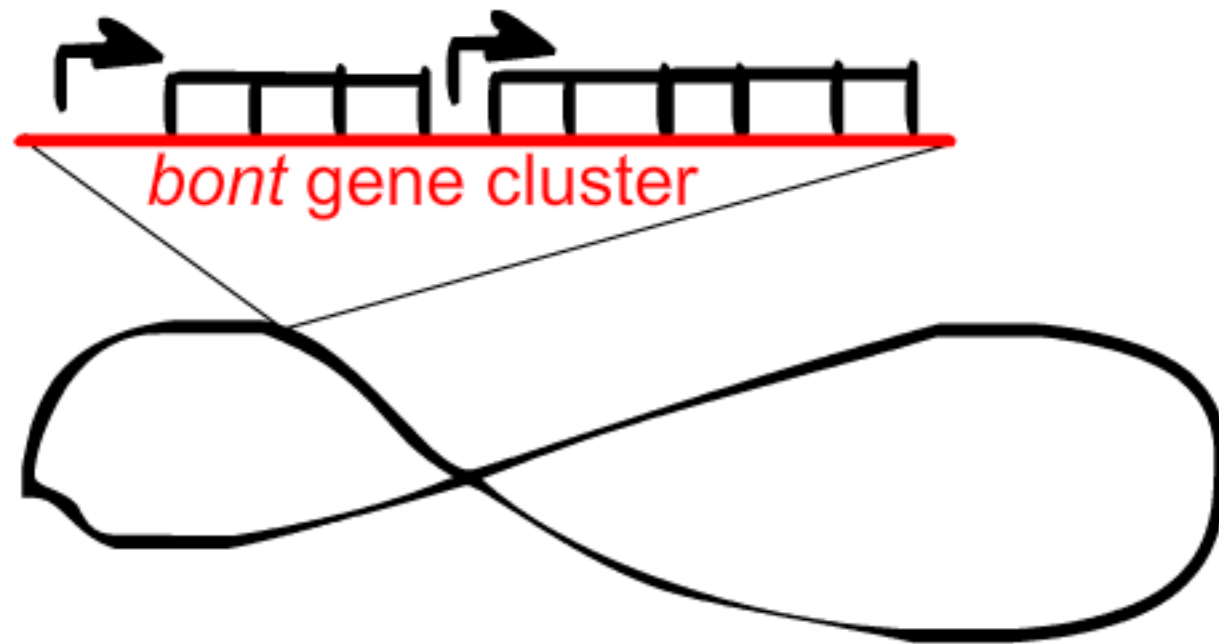


Case Study: *Clostridium botulinum*

Clostridium sporogenes

C. b. = one of the most important pathogens associated with food
C. s. = a harmless spore forming bacteria found in soil.

C.b. Strains produce one of seven known types of BoNT (A to G). Only those producing types A, B, E and F (rarely) cause botulism in humans (WHO, 2002).



Produces an Exotoxin or secreted bacterial toxin

"Treatment of Glabellar Frown Lines with C. Botulinum-A Exotoxin"

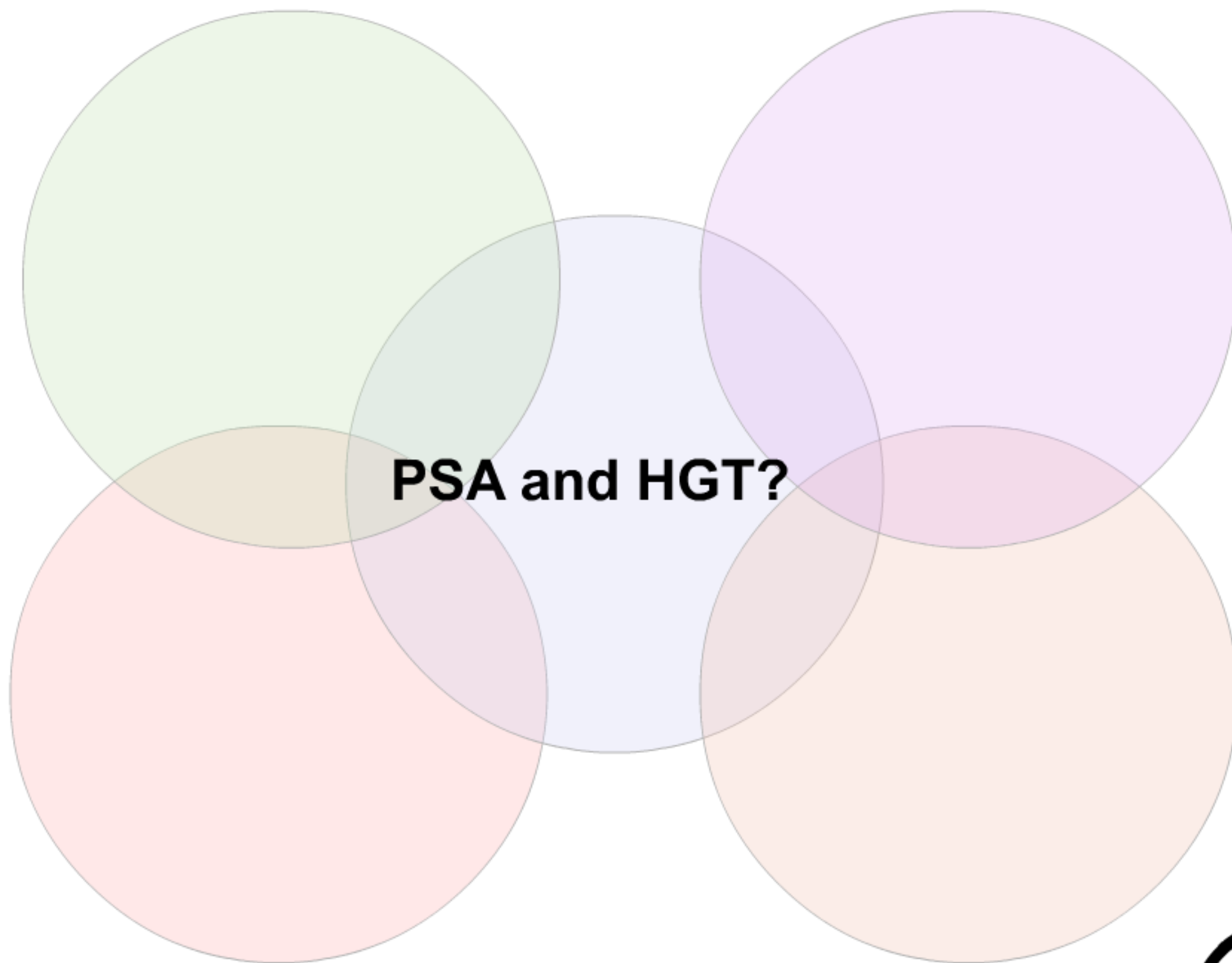


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bont on Chromosome
or plasmids
Antibiotic Resistance





PSA and HGT?



Antibiotic Resistance



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Case Study: *Pseudomonas syringae actinidiae*

PSA

Many *Pseudomonas* strains are harmless or even beneficial to plants (mutualists or symbionts).

PSA is a devastating pathogen destroying kiwifruit crops (parasite).



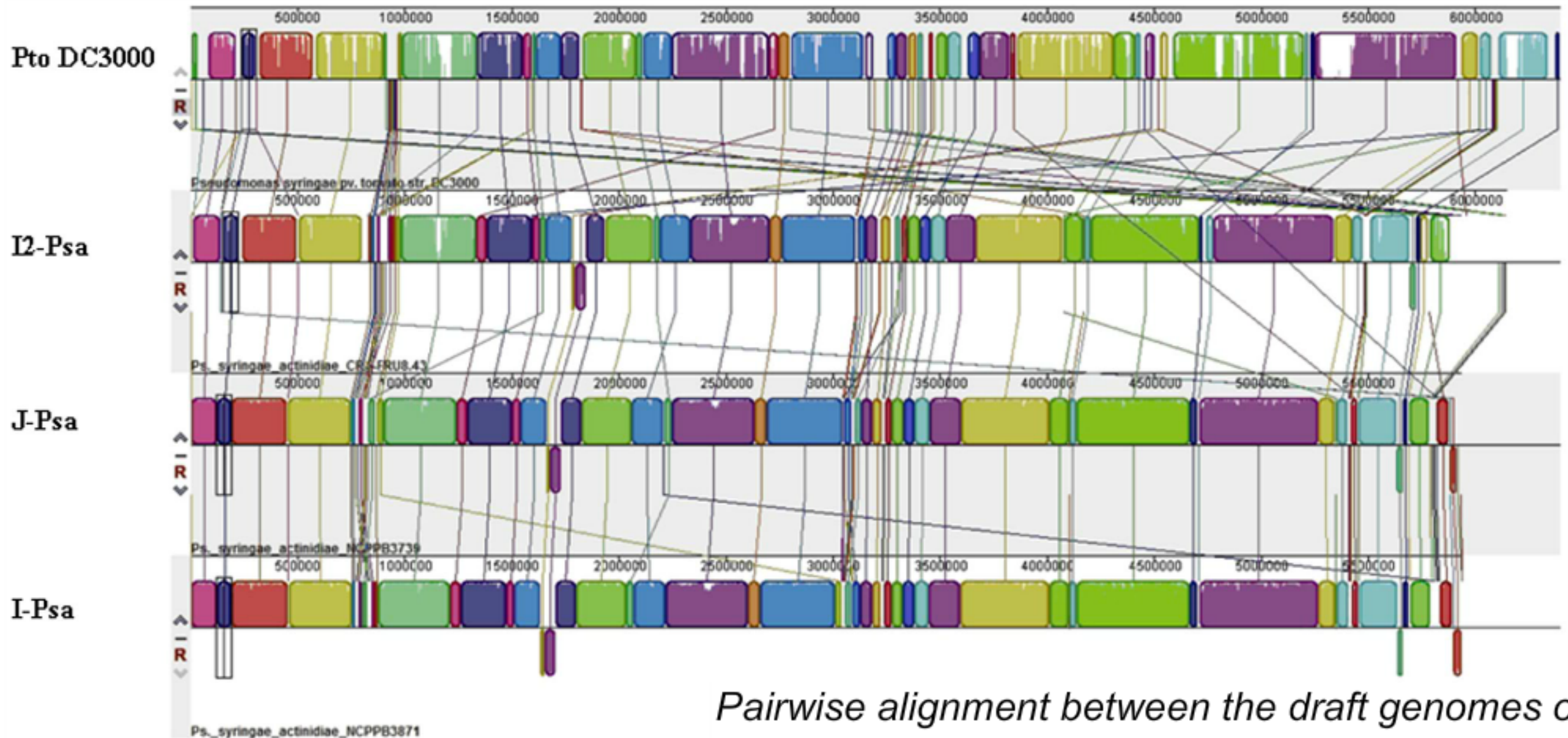
Antibiotic Resistance

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0027297>

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Case Study: *Pseudomonas syringae actinidae*



Pairwise alignment between the draft genomes of J-Psa, I-Psa and I2-Psa and the complete genome of P. s. pv. tomatoDC3000

Colored = similar across strains
White = specific to strain (HGT)

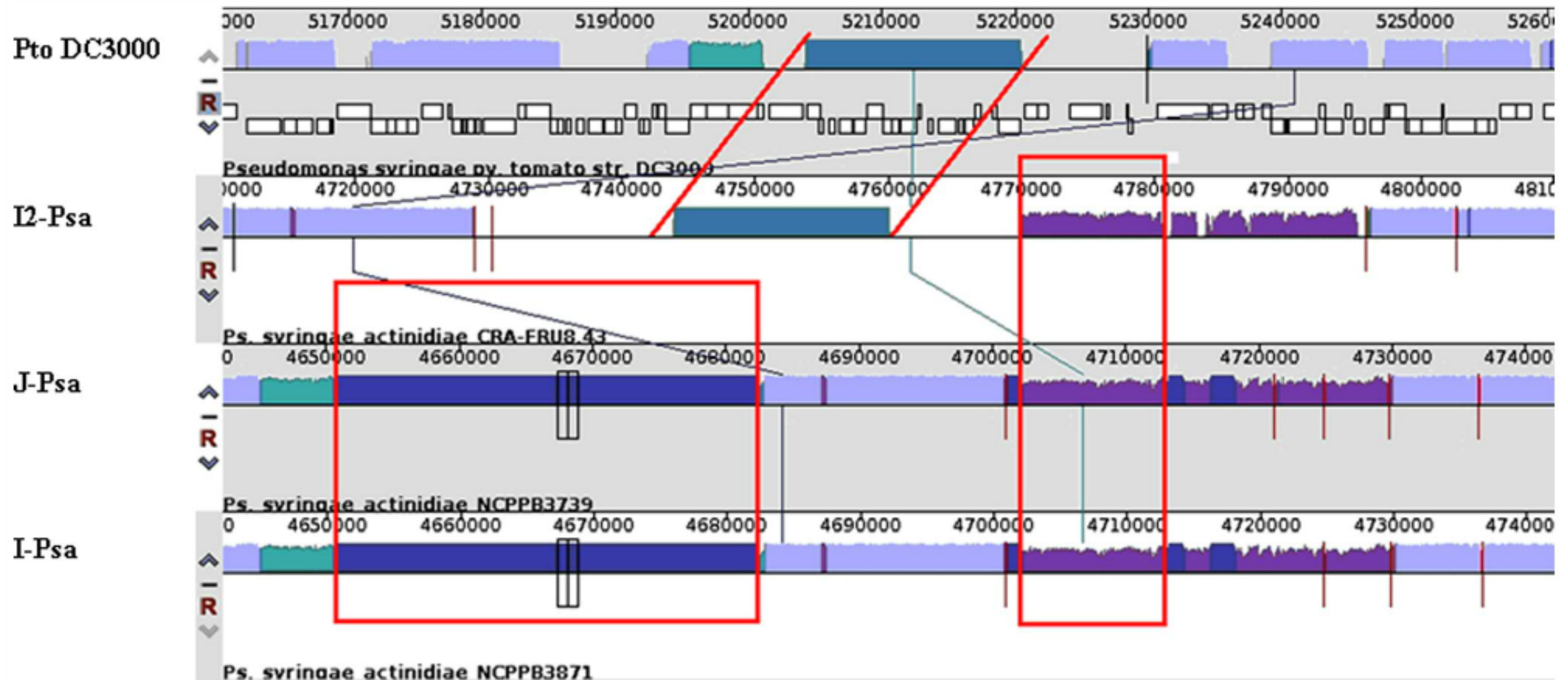
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next

Case Study: *Pseudomonas syringae actinidae*

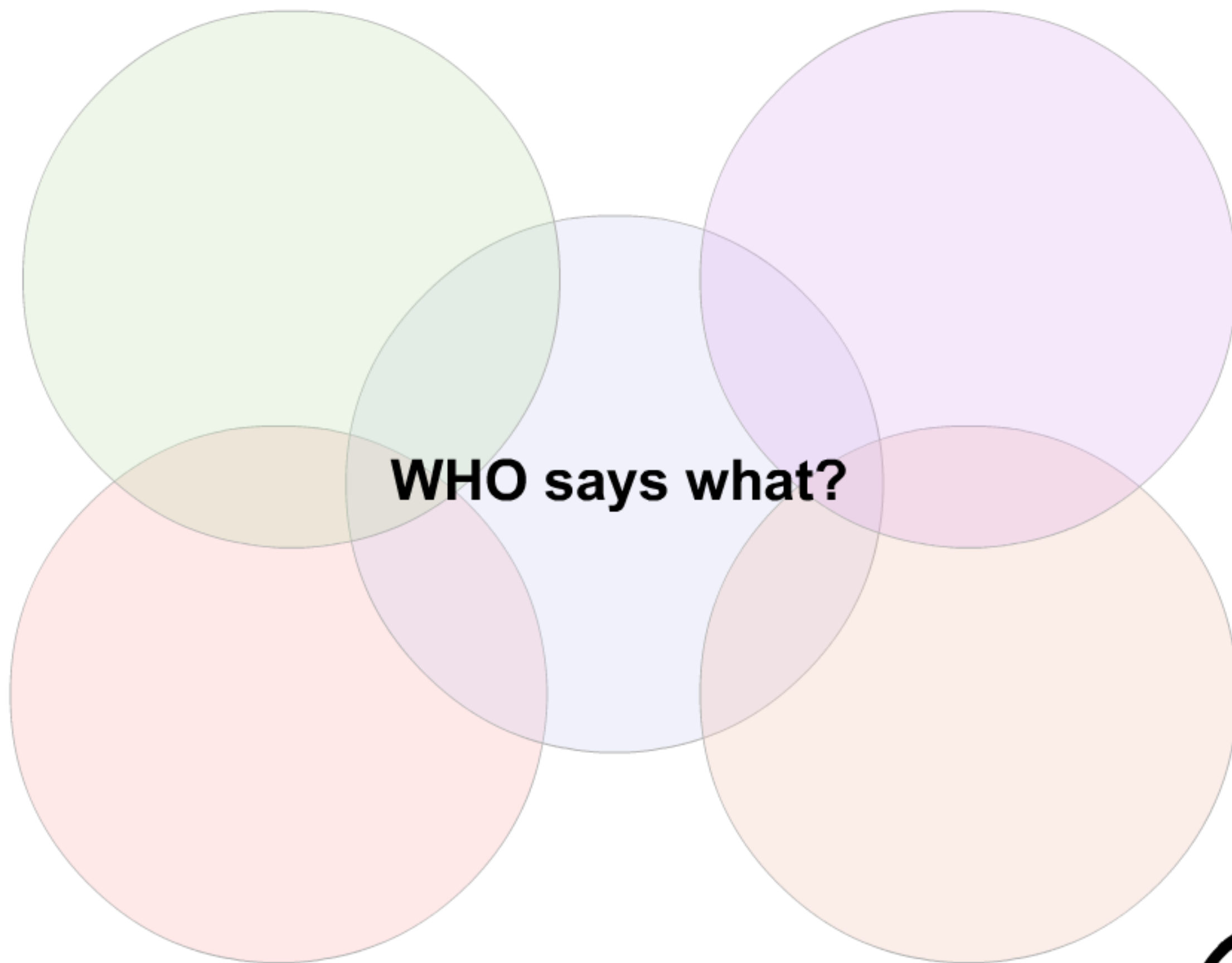
PSA



Representative part of the genome alignment between Psa strains and Pto DC3000 showing some variable regions.

closer look...





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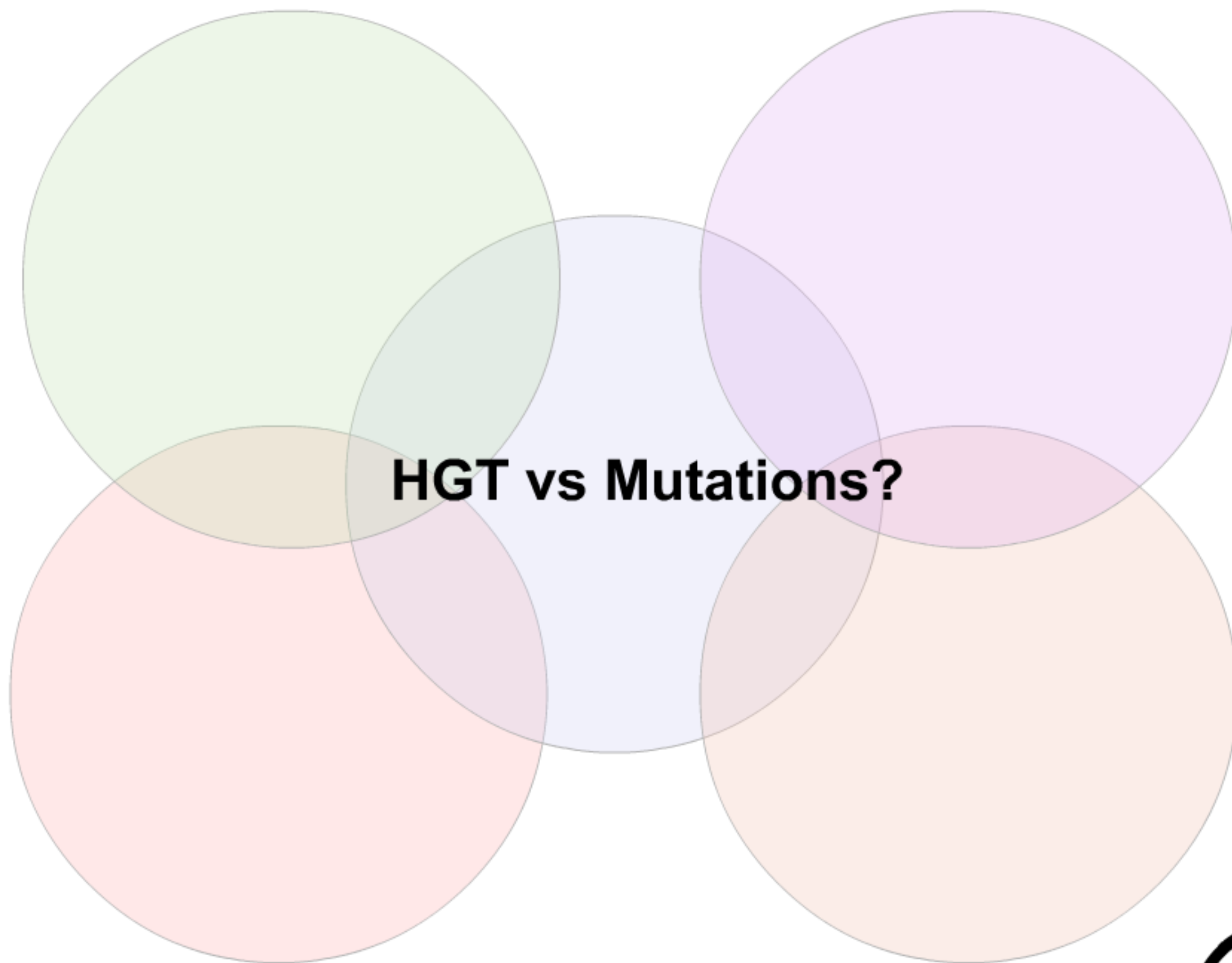


WHO Antibiotic Resistance Report

Infections:	Resistance in New Zealand:	Global region perspective:
Urinary tract infections, blood stream infections	<i>Escherichia coli</i> Resistance to third-generation cephalosporins 4.7% in blood 2.9% Urinary isolates <i>Escherichia coli</i> : Resistance to fluoroquinolones 7.5% Blood isolates 6.5% Urinary isolates	5/6 regions report nations with 50% resistance or more. 5/6 regions report nations with 50% resistance or more.
Pneumonia, blood stream infections, urinary tract infections	<i>Klebsiella pneumoniae</i> Resistance to third-generation cephalosporins 12.70% <i>Klebsiella pneumoniae</i> : Resistance to carbapenems 0%	6/6 regions report nations with 50% resistance or more. 2/6 regions report nations with 50% resistance or more.
Wound infections, blood stream infections	<i>Staphylococcus aureus</i> Resistance to methicillin (MRSA) 10.40%	5/6 regions report nations with 50% resistance or more.
Pneumonia, meningitis, otitis	<i>Streptococcus pneumoniae</i> : Resistance or non-susceptibility to penicillin 14.90%	6/6 regions report nations with 25% resistance or more.
Foodborne diarrhoea, blood stream infections	Nontyphoidal <i>Salmonella</i> (NTS): Resistance to fluoroquinolones 0.50%	3/6 regions report nations with 25% resistance or more.
Diarrhoea ("bacillary dysentery")	<i>Shigella</i> : Resistance to fluoroquinolones Not Available	2/6 regions report nations with 25% resistance or more.
Gonorrhoea	<i>Neisseria gonorrhoeae</i> : Decreased susceptibility to third-generation cephalosporins 3.20%	3/6 regions report nations with 25% resistance or more.

All data from the WHO ANTIMICROBIAL RESISTANCE Global Report on surveillance 2014





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Mutation rates are similar across all organisms.

Spontaneous mutation:

Chances of mutation in particular gene=
1 in 10 million per cell division ($1 * 10^{-7}$)
(sounds small...)

$2 * 10^{10}$ new cells in your intestine each day
(2 billion)

$(2 * 10^{10}) * (1 * 10^{-7}) = 2,000$ bacteria with
that gene mutated...



Most mutations have a negative effect on fitness and are quickly eliminated

**Neutral mutations
can persist**

**Beneficial mutations
are rare**





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