Why Don’t These Drugs Work Anymore?

Biosciences in the 21st Century

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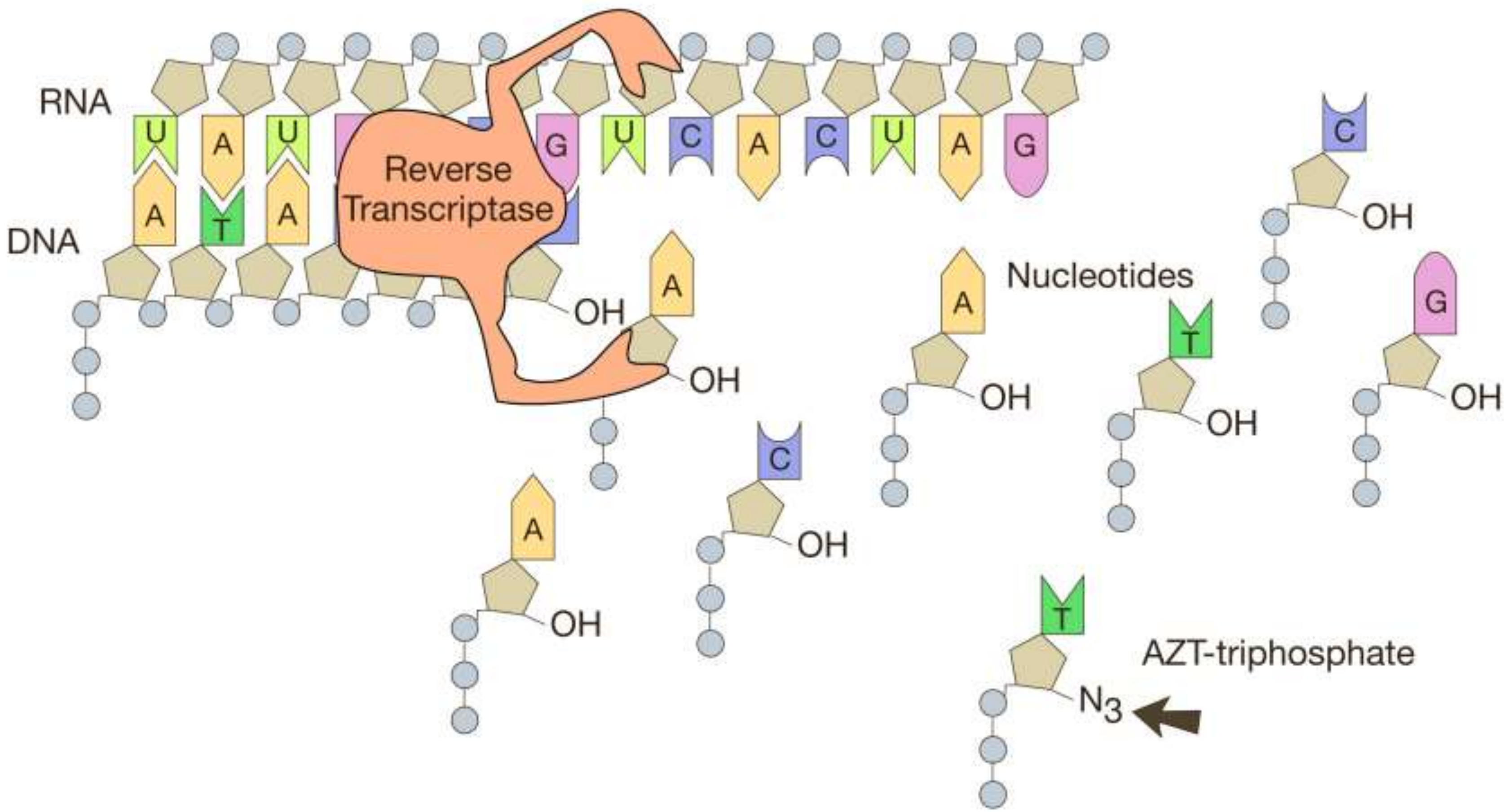
Outline

- Drug resistance: a case study
- Evolution: the basics
- How does resistance evolve?
  - Examples of “superbugs”
- Avoiding more widespread resistance

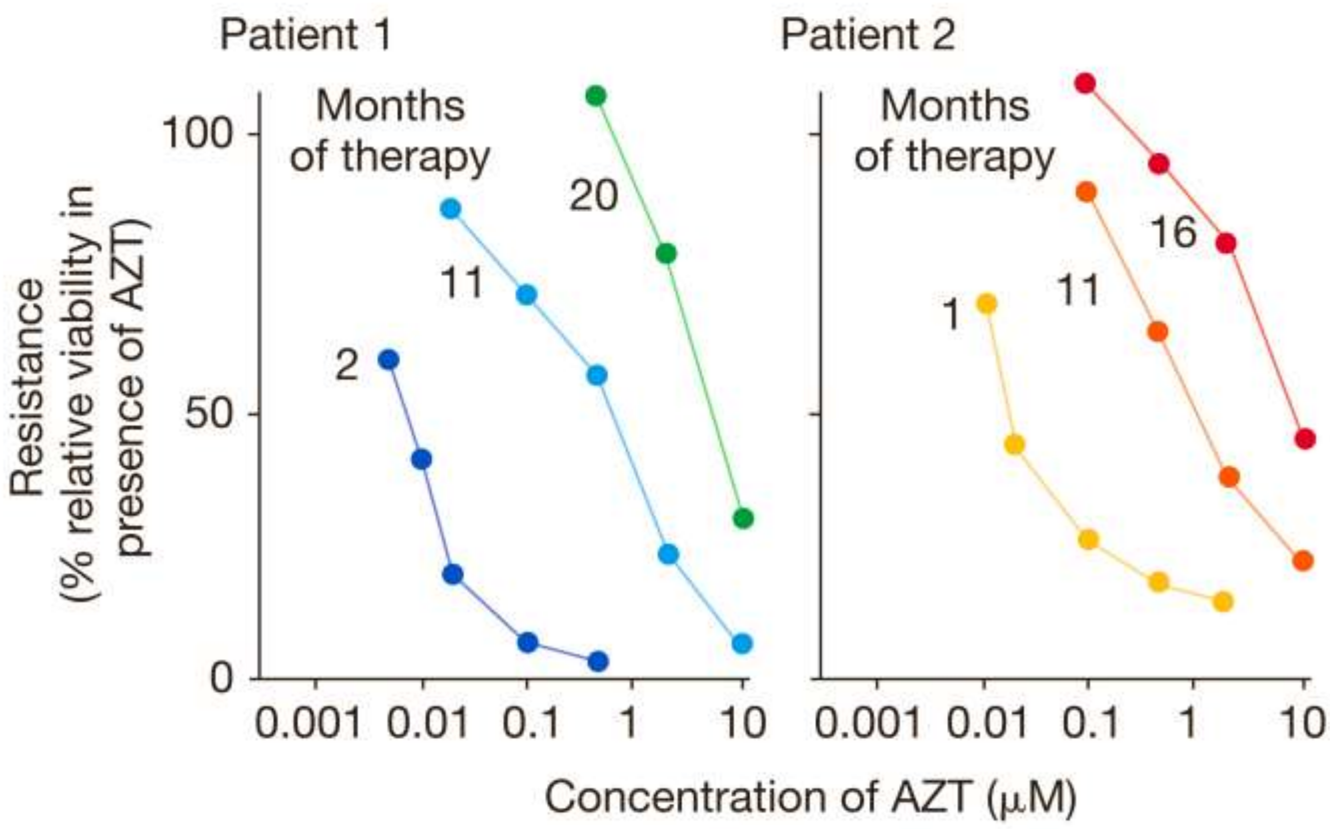
Drug resistance: a case study

- AZT (azidothymidine) approved as a treatment for HIV in 1987.



Drug resistance: a case study

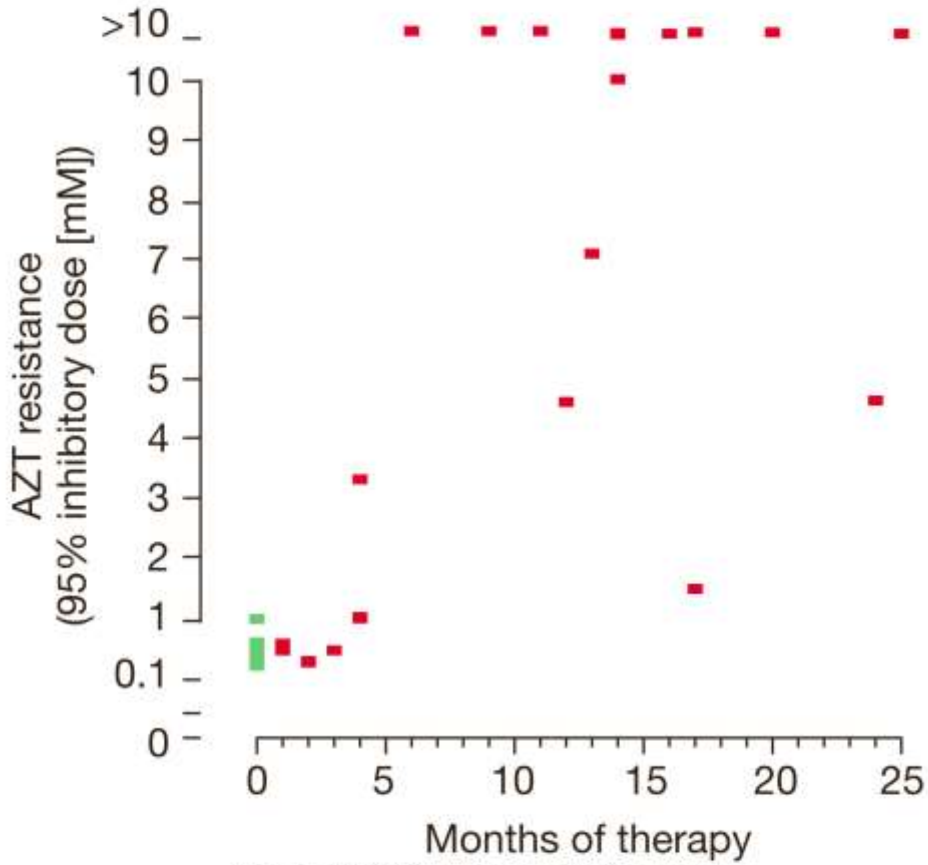
- After several months of treatment, higher concentrations of AZT were needed.



Drug resistance: a case study

- In most cases, patients became resistant to AZT within 6 months!

(b) Resistance in 39 patients, checked at different times



What happened?

Why might a drug stop working so quickly?

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What is evolution?

Evolution is a change in a population's allele frequencies over time.

Generation t		Generation t+1
aa		Aa
AA		Aa
AA		AA
Aa		Aa
AA	time	aa
AA		AA
AA		AA
aa		aa
AA		AA
Aa		Aa
70% A 30% a		60% A 40% a

What are the mechanisms of evolution?

1. Mutation: a change in DNA sequence, gene order, or chromosome number
   - Random
   - Increases genetic variation within populations
   - Types of mutations:
     - Point mutations
     - Insertions
     - Deletions
     - Gene duplications
     - Chromosomal inversions
     - Polyploidy

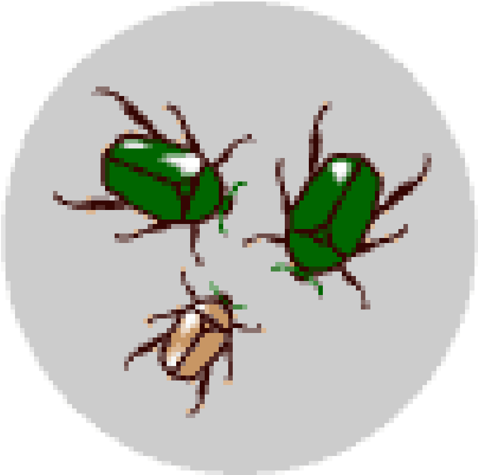


Figure: Univ. of Calif. Mus. of Paleontology's Understanding Evolution Site

What are the mechanisms of evolution?

2. <u>Gene flow (or migration)</u>: movement of genes between populations

- Increases genetic variation within populations
- Makes populations more similar to each other

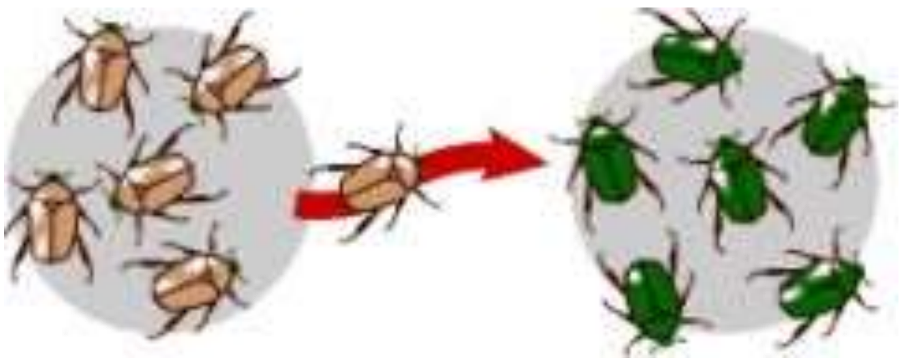


Figure: Univ. of Calif. Mus. of Paleontology's Understanding Evolution Site

What are the mechanisms of evolution?

3. Genetic drift: random changes in gene frequencies from one generation to the next (sampling error)

- Non-adaptive
- Decreases genetic variation within populations
- Makes populations more different from each other (divergence)
- Acts faster in small populations

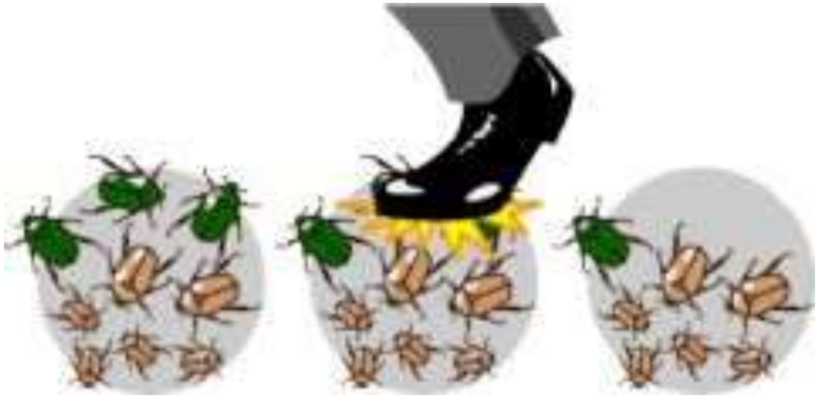


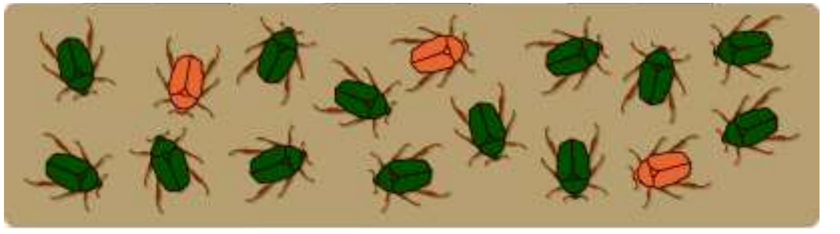
Figure: Univ. of Calif. Mus. of Paleontology's Understanding Evolution Site

What are the mechanisms of evolution?

4. Natural selection: differential reproductive success
   - Non-random
   - Not forward-looking, can only work with existing variation
   - Only adaptive mechanism of evolution

Figure: Univ. of Calif. Mus. of Paleontology's Understanding Evolution Site

Evolution by natural selection



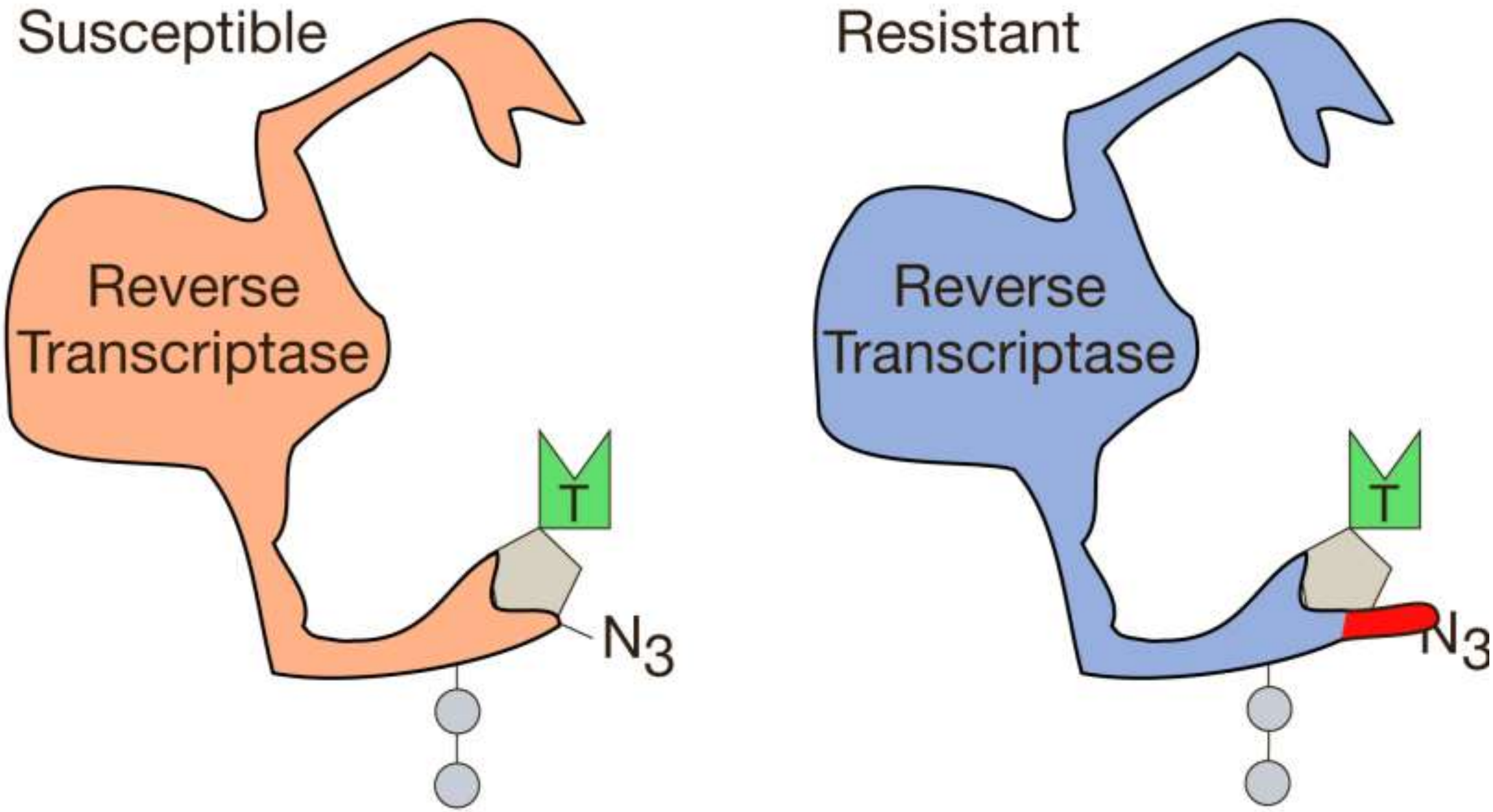
Ingredients needed for evolution by natural selection

- Variation in traits
- Inheritance
- Differential reproduction (natural selection)

End result: Traits that increase reproductive success increase in frequency in a population.

Figure: Univ. of Calif. Mus. of Paleontology's Understanding Evolution Site

Back to our case study: the evolution of resistance



Back to our case study: the evolution of resistance

Mutation **C**

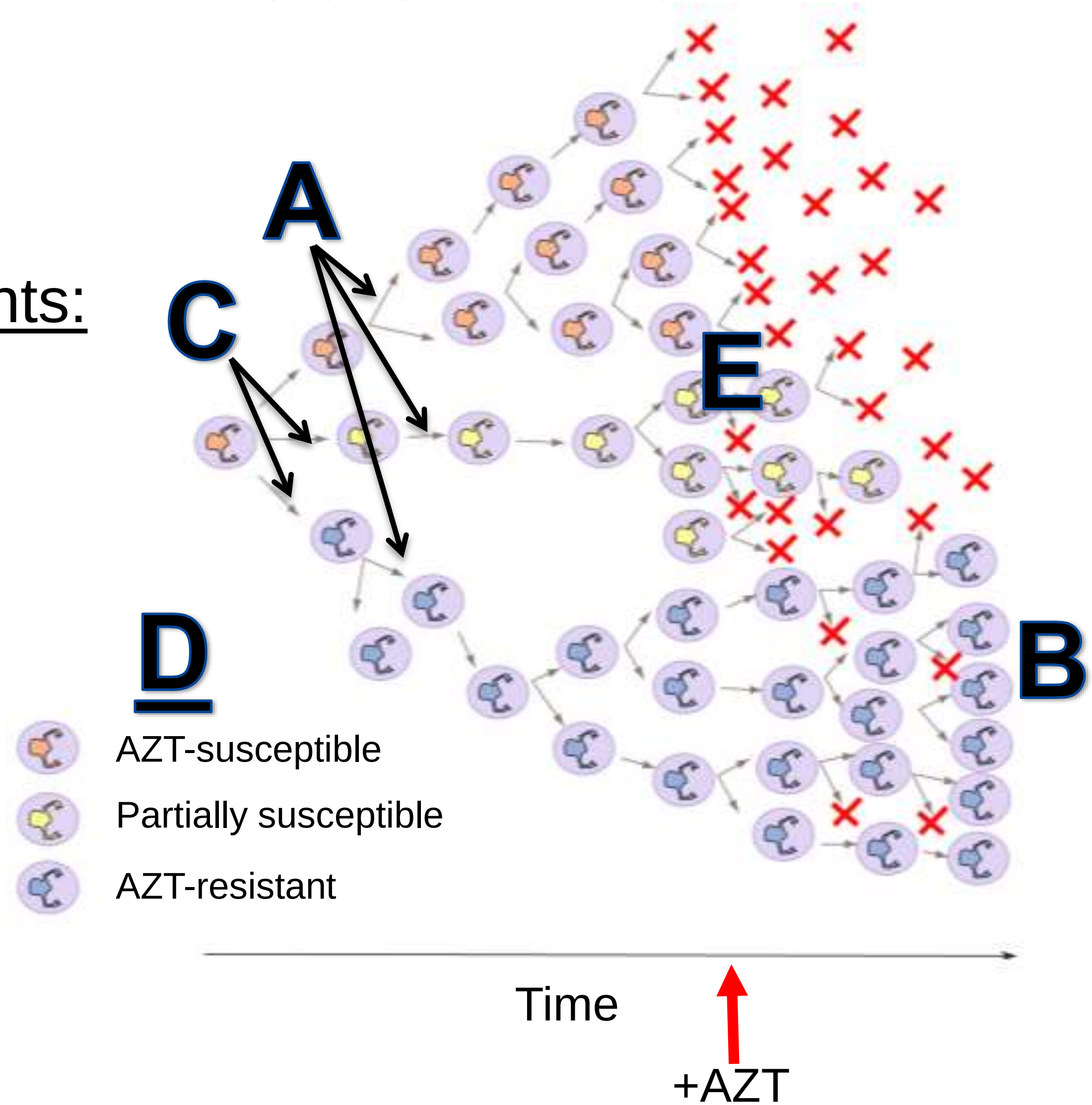
<u>Natural selection ingredients:</u>

Variation **D**

Inheritance **A**

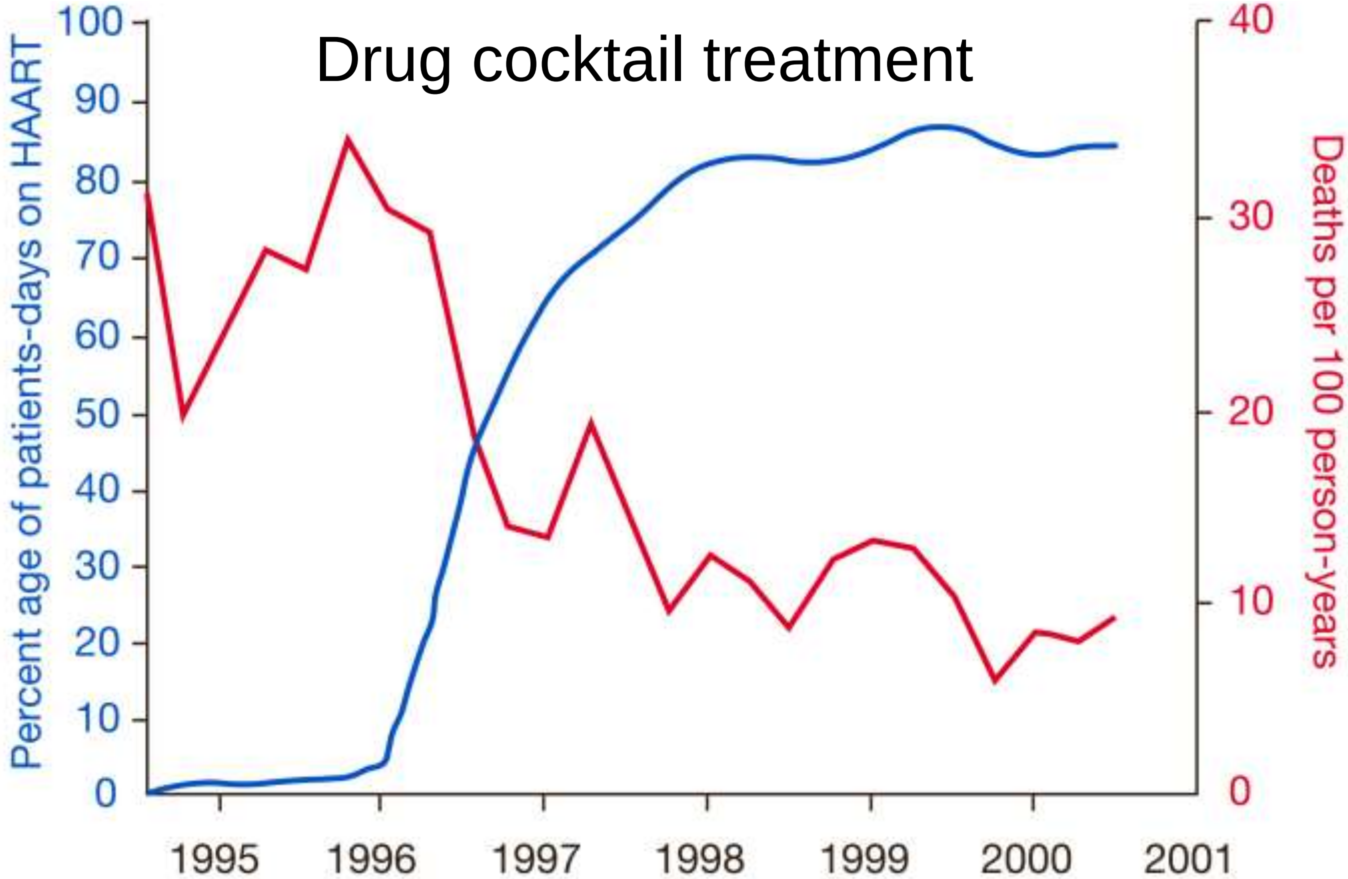
Differential reproductive success **E**

End result: AZT-resistant HIV strain **B**



Back to our case study: new treatments informed by evolution

- By understanding how resistance evolves, researchers could design new treatments.



Why are drug cocktails more effective?

- With a single drug, only 1 mutation can confer resistance.
  - Easy. HIV has large populations, a short generation time, and a high mutation rate.
- For resistance to drug cocktails,

- More mutations needed for resistance→ lower probability the mutations will occur together in one virion

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Important terms

- Antimicrobial: substances that kill or slow the growth of microbes
- Microbes: microscopic organisms including bacteria, viruses, parasites, and some fungi
- Antibiotic: drug developed to kill or slow the growth of bacteria

What “ingredients” are needed for the evolution of antimicrobial resistance by natural selection?

Evolution of resistance

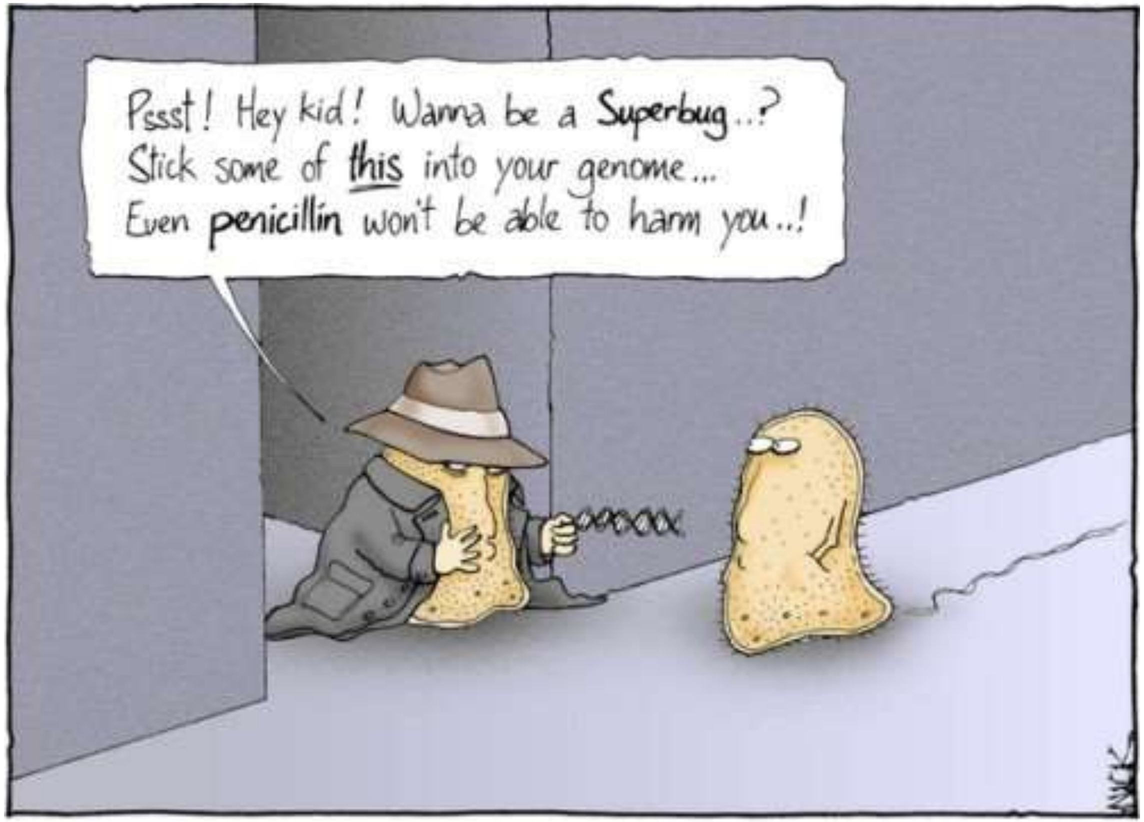
- Heritable variation for resistance
  - 30,000 year-old bacterial DNA recovered from Yukon permafrost
  - Genomic analyses identified genes for resistance to several antibiotics, including tetracycline and vancomycin (D’Costa et al. 2011, Nature)
  - So, resistant strains:
    - can pre-date use of the antimicrobial drug.
    - may arise by random mutation or even gene transfer after the drug is in use.

Evolution of resistance

- Differential reproduction
  - Widespread use of antibiotics creates **strong selection** for resistant strains.
    - Antibiotics over-prescribed by doctors
    - Antibiotics used in agriculture and commercial products
  - Use of *any* anti-microbial drug, not only antibiotics, creates strong selection for resistance.

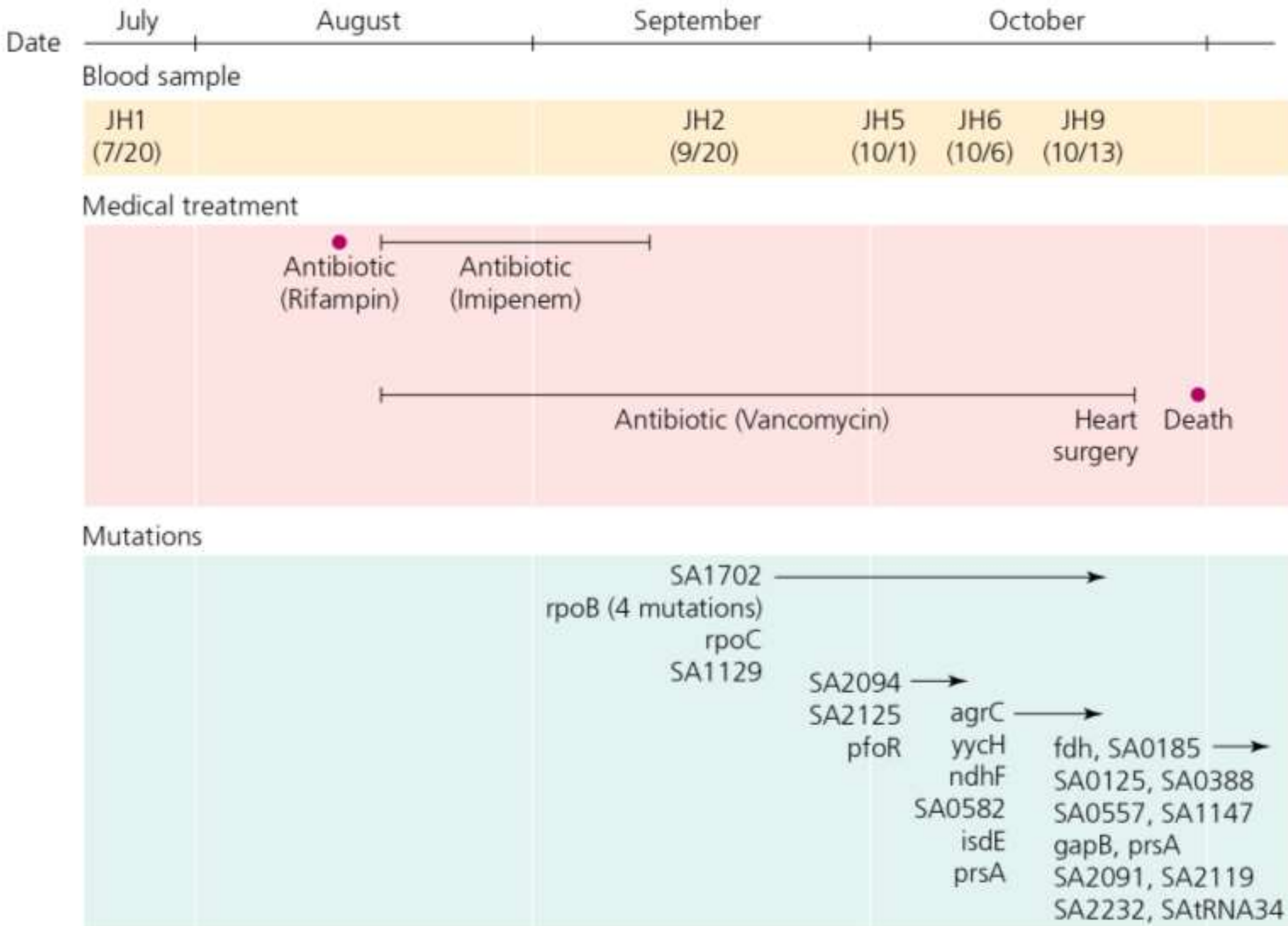
Evolution of resistance

- End result: Superbugs



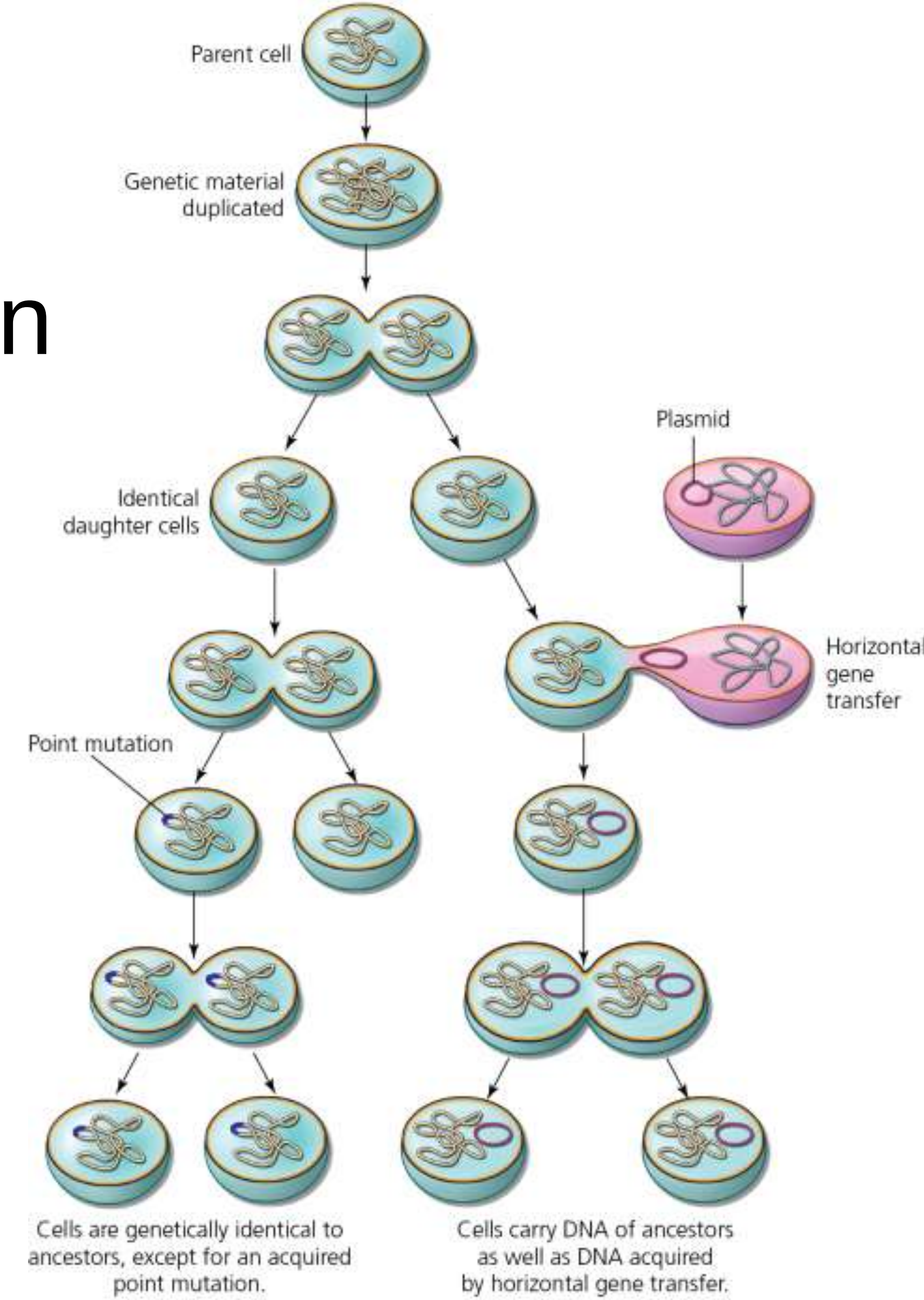
It was on a short-cut through the hospital kitchens that Albert
was first approached by a member of the Antibiotic Resistance.

Evolution of resistance: mutation by mutation



Evolution of resistance: a dangerous twist in the story

- Bacteria can also pick-up resistance genes through **horizontal gene transfer**



Antimicrobial resistance: general facts

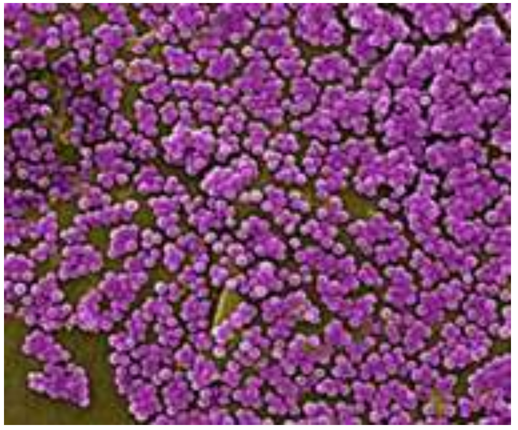
- Global concern
  - Long distance spread through travel and trade
- Longer illnesses, higher risks of death
  - Greater chance of spread when patients infectious for longer
- Increased healthcare costs
  - 5-10% U.S. hospital patients develop a resistant infection
  - $5 billion increase in annual healthcare costs!
- Growing problem
  - ~90,000 U.S. patients die each year vs. ~13,000 in 1992

World Health Organization (WHO)
U.S. National Institute of Allergy and Infectious Diseases (NIAID)

MRSA

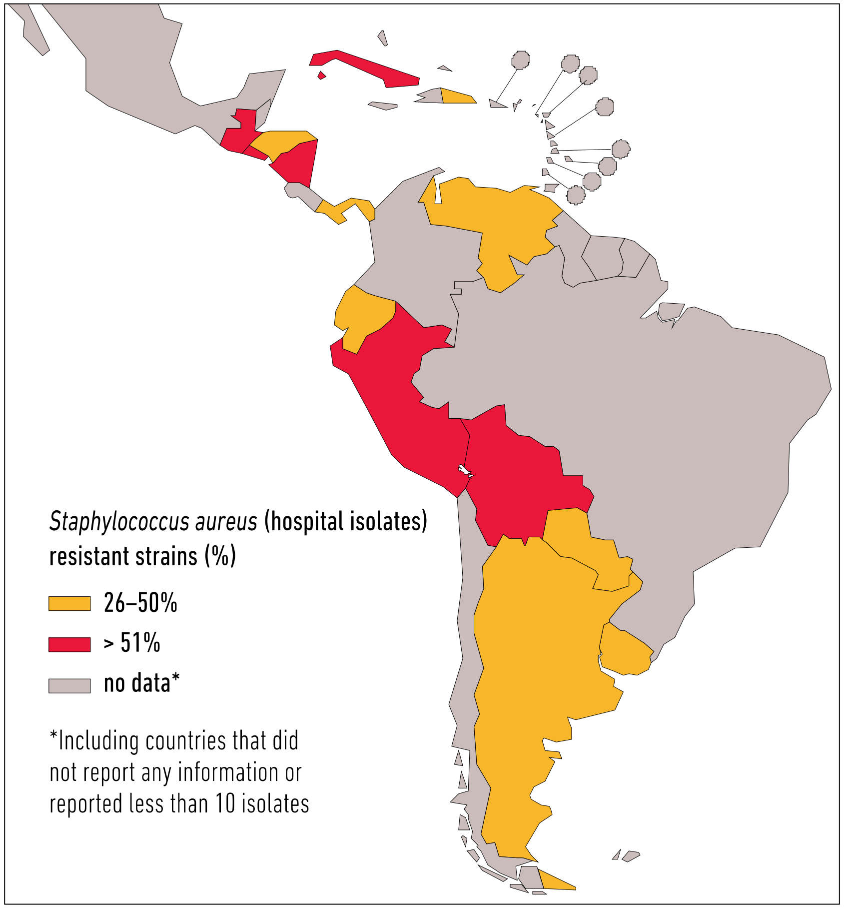
Methicillin-resistant *Staphylococcus aureus*

- Spread in hospitals and with close physical contact (e.g., among inmates, athletes)
- 33% worldwide have Staph, ~1% MRSA
- Painful skin conditions, even bacterial pneumonia and blood infections
- Can be fatal
- Resistant to entire class of penicillin-like antibiotics
- In 2002, vancomycin-resistant strain found



MRSA

Staphylococcus aureus **(hospital isolates): percentage of methicillin-resistant strains, 2007, Latin America and the Caribbean**



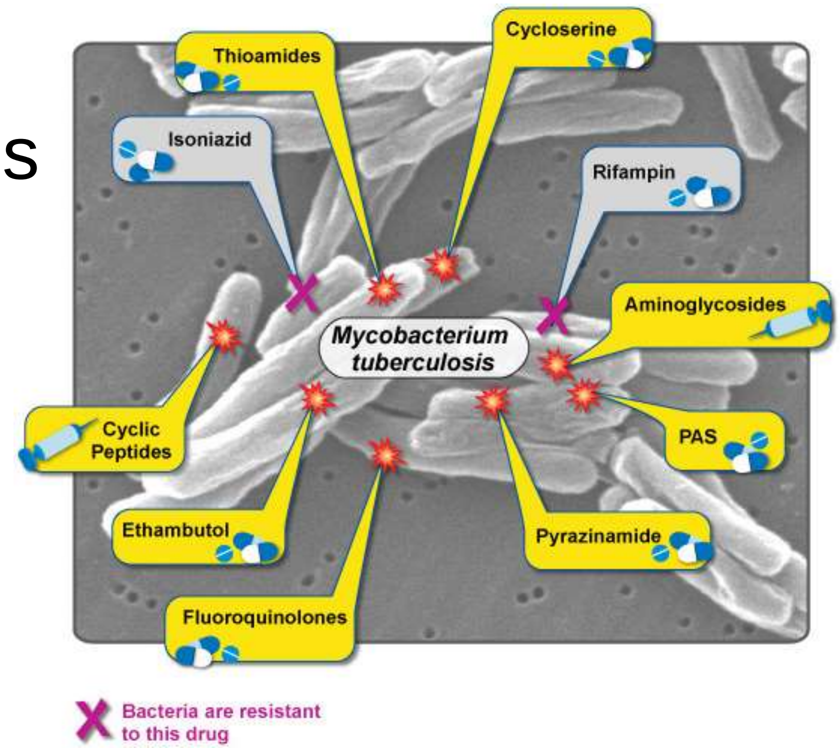
Adapted from: *Annual report on the antibiotic resistance monitoring/surveillance network, 2008*

Source: Latin American Resistance Surveillance Network, 2007. © PAHO HSD/CD 2011

MDR-TB

Multidrug-resistant Tuberculosis

- TB is major cause of death worldwide.
  - 2 million TB-related deaths each year
- 440,000 MDR-TB cases each year
  - 150,000 deaths
- 2nd line drugs have more side-effects, cost up to 100x more!

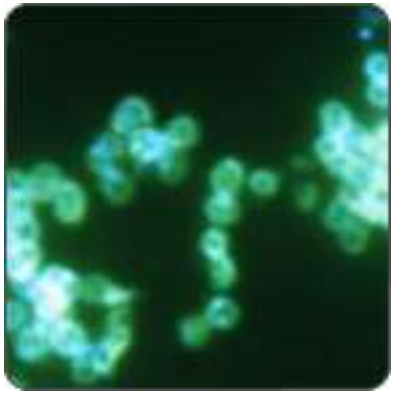


U.S. National Institute of Allergy and Infectious Diseases (NIAID)

Gonorrhea

Neisseria gonorrhoeae

- Sexually transmitted disease
- Bacterial
- ~700,000 new infections in U.S. each year
- Can lead to infertility in both sexes
- Can spread to blood and joints, potentially life-threatening
- Easily takes up DNA from other bacteria
- Resistant to all but one class of antibiotics
- Serious problem worldwide



Centers for Disease Control and Prevention (CDC)
U.S. National Institute of Allergy and Infectious Diseases (NIAID)

Malaria

- Caused by *Plasmodium spp.* protozoan
- Transmitted by mosquito
- Tropical and sub-tropical regions
- Fever, muscle & back pain, vomiting, anemia…
- Brain damage in children
- Nearly 1 million deaths each year
- Drugs used for treatment and for prevention
- Resistance to cheapest and most commonly used drugs is widespread
- Resistance to newer drugs is emerging

World Health Organization (WHO)
U.S. National Institute of Allergy and Infectious Diseases (NIAID)

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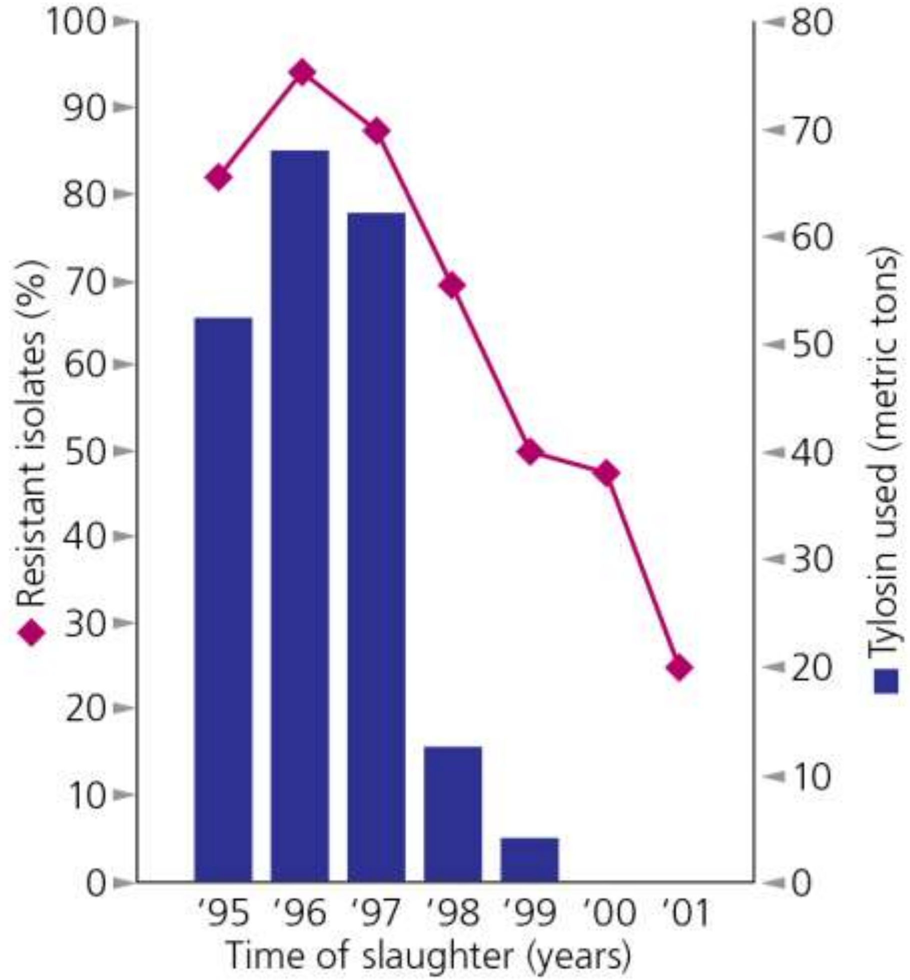
Avoiding more widespread resistance

1. Avoid contracting infections
2. Minimize transmission of resistant microbes
3. Improve use of antimicrobial drugs
   - Take only when appropriate (i.e., don’t take an antibiotic for the flu!)
   - Use antibacterial soaps/cleaners ONLY around people with weakened immune systems
   - Avoid broad-spectrum antibiotics if possible.
   - Take ALL of the medication
   - Reduce agricultural use of antibiotics

Why can reducing inappropriate use of antimicrobial drugs combat resistance?

- Resistance is sometimes costly for microbes.



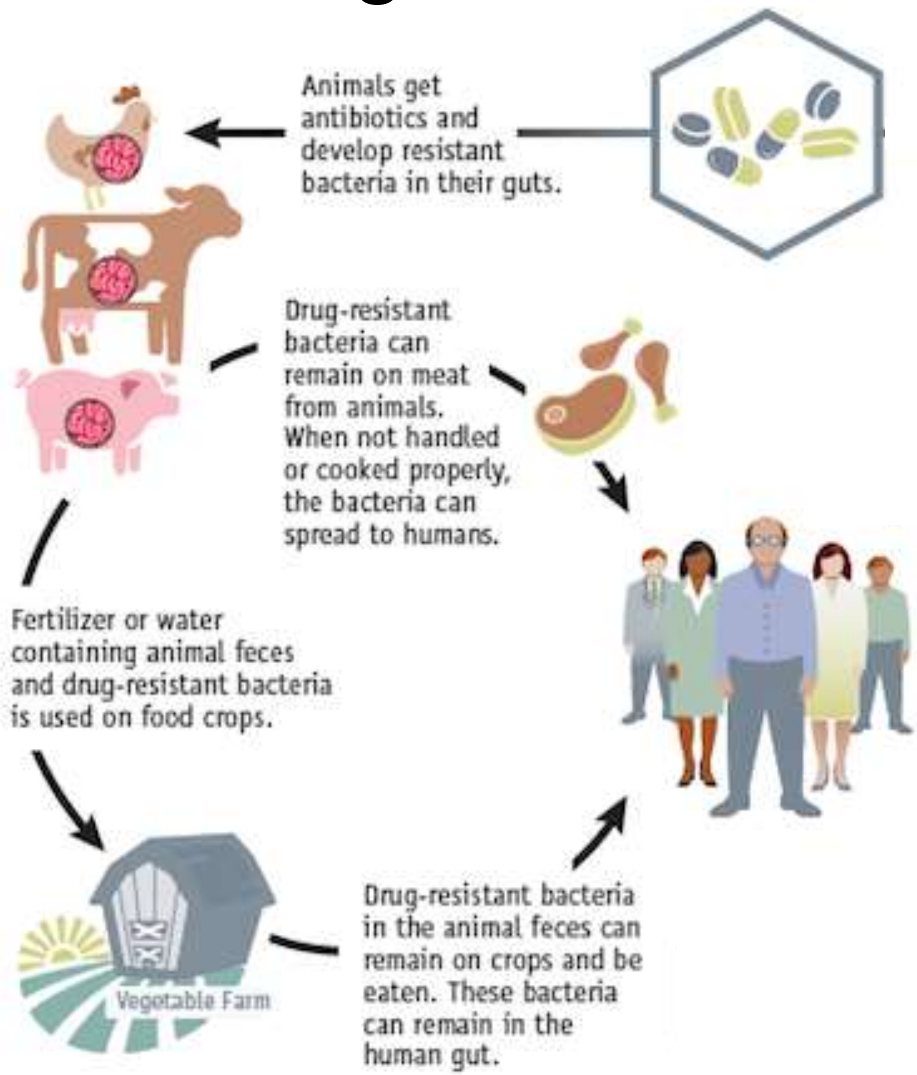
Why can reducing inappropriate use of antimicrobial drugs combat resistance?

- Use of specific antibiotics (not broad-spectrum)
  - Some antibiotics target a greater number of bacterial species.
  - Often used when diagnosis is unclear.
  - Why is it better to prescribe an antibiotic that targets fewer species?
    - Selection for resistance will act only on the species that are targeted by the drug.

Why can reducing inappropriate use of antimicrobial drugs combat resistance?

- Taking ALL of the medication increases the chance of exterminating the microbial population before resistance evolves.
  - If you stop early, you may get sick again or stay sick for longer.
  - Longer illness → more bacterial generations → greater chance of mutation for resistance arising
  - Even if resistant microbes DO arise, immune system may successfully fight them if population is small.
    - Stopping the drug lets the population grow larger.

Why can reducing inappropriate use of antimicrobial drugs combat resistance?



- Resistant bacteria escape livestock, spread to humans

Current research aims

- What is the mechanism of resistance?
- How do microbes acquire and pass on resistance genes?
- Development of better diagnostic tests to avoid the need for “broad spectrum” antibiotics
- Development of new drugs/vaccines

Key points

- Drug resistance is a serious problem worldwide.
- Understanding evolution is key to designing effective treatments and avoiding resistance in the first place.
- Evolution occurs by 4 mechanisms: mutation, gene flow, genetic drift, and natural selection.
- Mutation and genetic drift are random; natural selection is not.
- Evolution by natural selection requires: genetic variation, inheritance, differential reproduction.
- Natural selection can only work with the variation that is present. It cannot provide what is "needed."