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!
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 $t$
  - $70\%$ A
  - $30\%$ a
- Generation $t+1$
  - $60\%$ 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
What are the mechanisms of evolution?

2. **Gene flow (or migration):** movement of genes between populations
   - Increases genetic variation within populations
   - Makes populations more similar to each other
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.
Back to our case study: the evolution of resistance

Susceptible
Reverse Transcriptase

Resistant
Reverse Transcriptase

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Back to our case study: the evolution of resistance

Mutation

Natural selection ingredients:
Variation
Inheritance
Differential reproductive success

End result: AZT-resistant HIV strain

AZT-susceptible
Partially susceptible
AZT-resistant

Time
+AZT
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

Blood sample:
- July: JH1 (7/20)
- August: JH2 (9/20)
- September: JH5 (10/1) JH9 (10/13)
- October: JH6 (10/6)

Medical treatment:
- Antibiotic (Rifampin) in August
- Antibiotic (Imipenem) in August
- Antibiotic (Vancomycin) in October
- Heart surgery in October
- Death in October

Mutations:
- SA1702 rpoB (4 mutations)
- rpoC SA1129
- SA2094 SA2125 pfoR
- agrC yycH
- ndhF SA0582
- isdE SA1147
- gapB, prsA SA2091, SA2119
- SA2232, SATRNA34
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
Staphylococcus aureus (hospital isolates): percentage of methicillin-resistant strains, 2007, Latin America and the Caribbean

Staphylococcus aureus (hospital isolates) resistant strains (%)

- 26–50%
- > 51%
- no data*

*Including countries that did not report any information or reported less than 10 isolates

Adapted from: Annual report on the antibiotic resistance monitoring/surveillance network, 2008
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 $\rightarrow$ more bacterial generations $\rightarrow$ 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.”