Antibiotic Resistance: Super drugs for super bugs

September 26th, 2013

Amy Rohlfing, Harvard University BBS Program
Marina Santiago, Harvard University Chemical Biology Program
John Santa Maria, Harvard University Chemical Biology Program
Roadmap for the evening

1. Background on bacteria and antibiotics

2. How bacteria become antibiotic resistant

3. The hunt for new antibiotics
Outline Part I

• What are bacteria?
• How bacteria cause disease
• How antibiotics kill bacteria
Need for new antibiotics
Need for new antibiotics

- 17 common disease causing bacteria have antibiotic resistant versions
- 23,000 deaths/year from antibiotic resistant bacteria
- Some bacteria are resistant to nearly all antibiotics
Relative size of bacteria

Atom

Virus

Bacteria

Animal Cell

Period

Tennis Ball

Adult Female

0.1 nanometers

100 nanometers

1 micrometer

100 micrometers

1 millimeter

10 centimeters

1 meter

Thomas Splettstoesser, Wikimedia commons
Nossedotti, Brito, Wikimedia commons
MesserWoland, Szczepan 1990, Wikimedia commons
Bacterial vs. Animal cells

Animal Cell

- Nucleus
- Genetic material

Bacterial Cell

- Genetic material

Ivan Lanin, Wikimedia commons
Anatomy of a bacterium

- Cell Membranes
- Genetic Material (DNA)
- Flagellum
- Cell Wall
Anatomy of a bacterium

Gram negative

Gram positive

Y Tambe, Wikimedia commons
Anatomy of a bacterium

All parts are made up of different molecular building blocks

Cell Membranes

Genetic Material (DNA)

Flagellum

Cell Wall

Gene

Protein

Thomas Splettstoesser, Wikimedia commons
Dcirovic, Wikimedia commons
Anatomy of a bacterium

All parts are made up of different molecular building blocks

Genetic Material (DNA)
Flagellum
Cell Membranes
Cell Wall
Sugars
Anatomy of a bacterium

All parts are made up of different molecular building blocks

Cell Membranes
Genetic Material (DNA)
Flagellum
Cell Wall
Lipids
Summary so far…

- Antibiotic resistance is a growing public health issue
- Bacteria are small, single celled organisms
- DNA encodes information to make a protein
- Bacteria are surrounded by a flexible cell membrane and rigid cell wall
- Cell wall is essential and helps bacteria keep their shape
Questions?
Bacteria live and thrive in diverse environments
Importance of “good” bacteria

- Almost all surfaces colonized by bacteria: microbiome
Pathogens have arsenal of disease causing factors

- Adhesins
- Secretion System
- Flagellum
- Effectors
- Toxins

Adapted from Y Tambe, Wikimedia Commons
Accidental discovery of penicillin

Fleming, Br Journ Exp Pathol. 1929 June; 10(3): 226-236
What are antibiotics?

- Made by bacteria and fungi
- Why do bacteria produce antibiotics?
Antibiotics target essential bacterial processes

Cell Membranes  →  Replication of Genetic Material

Cell Wall

→  Production of proteins

→  Protein
How penicillin works
How penicillin works
Summary Part I

• Bacteria live and thrive in diverse environments all over the planet
• Pathogens have an arsenal of disease causing factors
• Antibiotics target processes essential to bacteria
• Antibiotic resistance is a growing public health concern

How do bacteria become resistant to antibiotics?
Questions?
Antibiotic Resistance: Super drugs for super bugs

September 26th, 2013
Amy Rohlfing, Harvard University BBS Program
Marina Santiago, Harvard University Chemical Biology Program
John Santa Maria, Harvard University Chemical Biology Program
Outline

• How antibiotics prevent cell machinery from working?
• How does antibiotic resistance work?
• Where does antibiotic resistance come from?
Outline

• **How antibiotics prevent cell machinery from working?**
• How does antibiotic resistance work?
• Where does antibiotic resistance come from?
Proteins are tiny machines that are made to perform one specific job

- **Enzymes** – Protein machines that perform one specific task for the cell with a chemical reaction

- **Target protein** – the protein that the antibiotic binds to and prevents from doing its normal job
Antibiotics fit in to their target proteins like a key in a lock

- Different keys fit different locks
- Different antibiotics fit inside different proteins
Antibiotics fit into their target proteins like a key in a lock

- Different keys fit different locks
- Different antibiotics fit inside different proteins
Antibiotics fit in to their target proteins like a key in a lock

• Different keys fit different locks
• Different antibiotics fit inside different proteins
Ampicillin binds in to a tiny pocket in its target protein

It fits exactly like a key in a lock
How can the bacteria rescue itself from the antibiotic?
Outline

• How antibiotics prevent cell machinery from working?
• **How does antibiotic resistance work?**
• Where does antibiotic resistance come from?
There are three ways for bacteria to become antibiotic resistant

1. Target resistance
2. Enzymatic degradation
3. Efflux pumps
There are three ways for bacteria to become antibiotic resistant

1. **Target resistance**
2. Enzymatic degradation
3. Efflux pumps
When a gene changes, the protein it codes for also changes.
Target Resistance - The bacteria’s DNA sequence changes to become resistant

- Every antibiotic targets some piece of machinery in the cell
- Penicillin targets the machinery that makes the cell wall
- The bacteria’s DNA sequence can change
- This results in the change of shape of the machinery
- Then, the antibiotic can not bind
- The enzyme can still do its job
Change the lock, the key will not fit
There are three ways for bacteria to get rid of antibiotics

1. Target resistance
2. **Enzymatic degradation**
3. Efflux pumps
Some bacteria have enzymes that can modify the antibiotic’s shape

- Enzymes can modify the shape of the antibiotic to make them harmless
- Change the key, will not fit in the lock
- Change the antibiotic, it will not fit in the protein it normally binds to
Beta-lactamases are a class of enzymes that modify amoxicillin.

- These enzymes break apart a piece of amoxicillin.
- The broken antibiotic does not bind to its target protein and it does not kill bacteria.
Scientists can test for beta-lactamases using an antibiotic called nitrocefin
There are three ways for bacteria to get rid of antibiotics:

1. Target resistance
2. Enzymatic degradation
3. Efflux pumps
Bacteria are like little houses

- Cold air in winter
- Dust
- Ground water
- Clean drinking water
- Fresh air
- Sewage
- Hot air in summer
Efflux pumps pump the antibiotic out of the cell

- There are proteins in the cell that can act like pumps
- Pump small molecules in and out of cell
- Some can pump out antibiotics
The efflux pump works in the cell membrane

Outside the Cell

Inside the Cell

Cell Membrane

Antibiotic

Wikimedia Commons Author: alexanderaloy and stargonzales. Original uploader was Star.gonzales at en.wikipedia
The efflux pump works in the cell membrane
Summary 1 and Questions?

- There are three ways for bacteria to resist antibiotics
  1. Target resistance
  2. Enzymatic degradation
  3. Efflux pumps
Outline

• How antibiotics prevent cell machinery from working?
• How does antibiotic resistance work?
• **Where does antibiotic resistance come from?**
Where does antibiotic resistance come from?

- Evolution/Selection
- Transfer of antibiotic resistance genes from another bacteria
Where does antibiotic resistance come from?

• **Evolution/Selection**

• Transfer of antibiotic resistance genes from another bacteria
Evolution does not have a direction
Evolution is a change in the characteristics of a population.

- Before the industrial revolution in England, there were more light-colored peppered moths than dark.
- They were harder to see on lichen-colored trees.
Evolution is a change in the characteristics of a population

Selection – A condition where a fraction of the population has an advantage in growth, reproduction, or survival.

Industrial Revolution:
Lichens died

Improved environment:
Lichens grew back

Wikimedia Commons Source: Photos taken by Olaf Leillinger
Evolution is a change in the characteristics of a population

Selection – A condition where a fraction of the population has an advantage in growth, reproduction, or survival

Industrial Revolution: Lichens died

Improved environment: Lichens grew back

Wikimedia Commons Source: Photos taken by Olaf Leillinger
Bacteria can evolve to be better at living on human cells

In the wild

In the body

Proteins grip human cells better
Salmonella is one type of bacteria that does this.
Evolution can also happen on the molecular level

Gene

Gene

There are natural variations in the shape of proteins because of differences in the genes

- Some proteins will bind to the antibiotics less well naturally
- They will be more likely to survive antibiotic treatment

Protein Target of the Antibiotic: Select for Proteins That Do Not Fit the Antibiotic: Antibiotic resistant bacteria!
Summary 2 and Questions

• Evolution is a change in the characteristics of the population
• A selection is a condition that gives some members of a population an advantage in growth, reproduction, or survival
• Evolution can happen on an animal, bacterial, or molecular level
Where does antibiotic resistance come from?

• Evolution/Selection

• **Transfer of antibiotic resistance genes from another bacteria**
Transfer of antibiotic resistance genes from more resistant bacteria

- There are two main ways to do this:
  - Phage – a virus that attacks bacteria – can transfer genes to other bacteria
  - Conjugation – transfer of genes from bacteria to bacteria
Transfer of antibiotic resistance genes from more resistant bacteria

• There are two main ways to do this:
  – **Phage** – a virus that attacks bacteria – can transfer genes to other bacteria
  – Conjugation – transfer of genes from bacteria to bacteria
Viruses can attack people, but they also attack bacteria

Avian Flu Virus

T4 phage
The normal viral life cycle

1.

2.

3.
Sometimes, the virus accidentally takes a bacterial gene instead

1. 2. 3. 4. 5. 6.
This happens relatively rarely

• For every 100,000,000 phage that attack a cell, only one will transfer a gene
• There are thousands of genes per cell
• The probability that it will transfer an antibiotic resistance gene is very low
• But, there are 100,000,000,000,000 bacteria in your body, so it is not as unusual as you might think
Transfer of antibiotic resistance genes from more resistant bacteria

• There are two main ways to do this:
  – Phage – a virus that attacks bacteria – can transfer genes to other bacteria
  – **Conjugation** – transfer of genes from bacteria to bacteria
Conjugation – The transfer of DNA by direct cell to cell contact

- Bacterial equivalent of sexual reproduction
- Pilus – tube that a cell produces that attaches to another bacterium to transfer DNA
- The DNA that is transferred could contain an antibiotic resistance gene
- **Now the recipient cell will be antibiotic resistant!**
Conclusion

• These are the weapons that bacteria are bringing to the battle.
• What can we do to fight back?

1.  
2.  
3.  
4.  
5.  
6.  

Antibiotic
Questions?
Super Drugs for Superbugs

September 26th, 2013
Amy Rohlfing, Harvard University BBS Program
Marina Santiago, Harvard University Chemical Biology Program
**John Santa Maria**, Harvard University Chemical Biology Program
…Where we left off

• Bacteria are small organisms that can cause infections. We can fight back with antibiotics.

• Bacteria can become resistant to antibiotics through different mechanisms

• It’s clear that we need new antibiotics…
Finding new antibiotics

- What do antibiotics look like?
- Where can we find new antibiotics?
- How do we find them?
What do antibiotics look like?

Tetracycline

Vancomycin

Penicillin G

Daptomycin

Kanamycin

Antibiotics are small molecules
Useful antibiotics have several key properties

1) Hits an important bacterial process

Penicillin G
Useful antibiotics have several key properties

1) Hits an important bacterial process

2) Is effective and specific for its intended target (non-toxic!)

Penicillin G
Useful antibiotics have several key properties

1) Hits an important bacterial process

2) Is effective and specific for its intended target (non-toxic!)

3) Resistant to degradation by bacteria or environment

Penicillin G
Useful antibiotics have several key properties

1) Hits an important bacterial process

2) Is effective and specific for its intended target (non-toxic!)

3) Resistant to degradation by bacteria or environment

4) Can get into and is well-tolerated by the body (easy dosing!)

Penicillin G
Useful antibiotics have several key properties

1) Hits an important bacterial process

2) Is effective and specific for its intended target (non-toxic!)

3) Resistant to degradation by bacteria or environment

4) Can get into and is well-tolerated by the body (easy dosing!)

5) Can be prepared in large quantities/cheaply
Many antibiotics come from Nature

FDA Approved Antibiotics 1981-2005

- Natural (9.2%)
- Modified from Nature (58.7%)
- Man-made (32.1%)

We’ve already seen an example of a naturally-produced antibiotic

Penicillin G
Pathogenic Agrobacterium tumefaciens causes crown gall disease
Agrobacterium radiobacter K84 makes agrocin 84 to kill disease-causing bacteria

Agrobacterium radiobacter K84 is used to protect plants and prevent disease.

Antibiotics are often made in very unique environments.
Scientists are on the hunt for more natural antibiotics

- Amass collections of organisms from diverse environments and look to see if they make useful small molecules
Scientists are on the hunt for more natural antibiotics

- Amass collections of organisms from diverse environments and look to see if they make useful small molecules
- Using genome sequencing, we can predict the antibiotics that will be made by bacteria or fungi
The majority of antibiotics we use are Nature-inspired, but human-modified

**FDA Approved Antibiotics 1981-2005**

- Natural (9.2%)
- Modified from Nature (58.7%)
- Man-made (32.1%)

We Can Chemically Improve Existing Antibiotics

Cephalosporin C from the fungus *Acremonium* discovered in 1948
We can chemically improve existing antibiotics

2\textsuperscript{nd} generation: Loracarbef (longer-lasting)
We can extensively tailor existing antibiotics

5<sup>th</sup> generation: Ceftobiprole (resistant to bacterial degradation, hits resistant bacterial enzyme), FDA approval 2010
Some antibiotics don’t come from Nature

FDA Approved Antibiotics 1981-2005

- Natural (9.2%)
- Modified from Nature (58.7%)
- Man-made (32.1%)

Salvarsan was the first synthetic antibiotic
discovered in 1909 by Sahachiro Hata & Paul Ehrlich.
We can modify synthetic antibiotics to prevent their efflux
We can modify synthetic antibiotics to prevent their efflux
Summary #1

• Nature is our richest source of antibiotics
  – Most new antibiotics are derived from old ones, but this process is difficult and does not substantially change how the drug works…

• Good antibiotics have key properties
  – They need to be safe & effective!

How do we find good antibiotics?
Scientists use two broad strategies to look for antibiotics:

Target-Based Screening
Scientists use two broad strategies to look for antibiotics

Target-Based Screening

Whole Cell Screening
Each approach has its own pros/cons
Each approach has its own pros/cons

**Goal:** Inactivate enzyme

Know target of small molecule already

Can it get into bacteria?
Each approach has its own pros/cons

**Target-Based Screening**
- Goal: Inactivate enzyme
- Know target of small molecule already
- Can it get into bacteria?

**Whole Cell Screening**
- Goal: Kill bacteria
- Target of small molecule unknown
- We know it can get into bacteria
If it’s so easy to kill bacteria, how come we don’t have more antibiotics?

• Case study:
  – One prominent pharmaceutical company analyzed 530,000 compounds for antibiotic activity
  • 67 experiments using purified proteins and 3 using whole bacteria cells
  • They found 5 potential leads (.0009% success rate)
  • This cost them >$70 million and took 6 years
  • Many companies did the same!

Why did they fail? How can we fix this?

• They didn’t look at the right small molecules

• They were trying to target the wrong proteins

• They were looking for a single molecule to accomplish a tough job

There’s more than one way to skin a cat

Combination therapies
Antivirulence compounds
Antibiotics may not have to kill bacteria…

Virulence factors are required to establish an infection in a host

Can block using antivirulence compounds (controversial)
Pilicide prevents *E. coli* from sticking to human cells

Source: Aberg & Almqvist Org. Biomol. Chem. 2007
Two drugs can be better than one

Block **enzymatic degradation**

Augmentin is a combination strategy to prevent antibiotic degradation by beta-lactamases.

Amoxicillin

Target cell wall
Cause bacterial death
Augmentin is a combination strategy to prevent antibiotic degradation by beta-lactamases.
Augmentin is a combination strategy to prevent antibiotic degradation by beta-lactamases.
Augmentin is a combination strategy to prevent antibiotic degradation by beta-lactamases
Augmentin is a combination strategy to prevent antibiotic degradation by beta-lactamases.

Amoxicillin

Target cell wall
Cause bacterial death

Clavulanic acid

Enzyme

STOP
Synthetic lethality may provide new targets for combinations

• A one-two punch

<table>
<thead>
<tr>
<th>Protein 1</th>
<th>Protein 2</th>
<th>Bacteria lives?</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>✨</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>✨</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>✨</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>☠️</td>
</tr>
</tbody>
</table>

Greatly expands potential targets, but now we need drugs to block these new targets
Antibiotic resistance is a complex problem.

Antibiotics

- Bacteria
- Doctors & Patients
- Government
- Researchers

$
Summary #2

• Large scale efforts by major pharmaceutical companies have failed to identify new antibiotics – it’s hard!

• Recent scientific advances can help us tackle antibiotic resistance
  – Alternative strategies for antibiotics
    • Combination therapies
    • Antivirulence
    • Combating resistance mechanisms
  – New targets
    • Synthetic lethality
Thank you!

*SITN would like to acknowledge the following organizations for their generous support.*

**Harvard Medical School**
Office of Communications and External Relations
Division of Medical Sciences

The Harvard Graduate School of Arts and Sciences (GSAS)

The Harvard Graduate Student Council (GSC)

The Harvard Biomedical Graduate Students Organization (BGSO)

The Harvard/MIT COOP
It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.
Importance of “good” bacteria

- Almost all surfaces colonized by bacteria
- Commensals protects against disease

Khosravi, Mazmanian. Curr Op Micro 2013. 16: 221-227
Importance of “good” bacteria

- Almost all surfaces colonized by bacteria
- Commensals protects against disease
- Commensals provides nutrients

Antibiotics target essential bacteria processes

Cell Wall Synthesis
- Beta Lactams
  - Penicillins
  - Cephalosporins
  - Carbapenems
  - Monobactams
- Vancomycin
- Bacitracin

Folate synthesis
- Sulfonamides
- Trimethoprim

Nucleic Acid Synthesis
- DNA Gyrase
  - Quinolones
- RNA Polymerase
  - Rifampin

Cell Membrane
- Polymyxins

30S subunit
- Tetracyclines
- Aminoglycosides

50S subunit
- Macrolides
- Clindamycin
- Linezolid
- Chloramphenicol
- Streptogramins

Protein Synthesis

©2011 TheMedSchool.com

Kendrick Johnson, Wikimedia Commons