Lecture 18

Evolution of antimicrobial resistance
The History of Medicine

- 2000 BC – Here, eat this root.
- AD 1000 – That root is heathen. Here, say this prayer.
- 1850 – That prayer is superstition. Here, drink this potion.
- 1920 – That potion is snake oil. Here, swallow this pill.
- 1945 – That pill is ineffective. Here, take this penicillin.
- 1955 – Oops...bugs mutated. Here, take this tetracycline.
- 1960–1999 – 39 more “oops”... Here, take this more powerful antibiotic.
- 2000 – The bugs have won! Here, eat this root.

—Anonymous

Society’s ongoing struggle against infectious disease.
Today:

1. History of antimicrobials

2. Evolution of antimicrobial resistance: “natural” selection in action

3. We’re not necessarily going to hell in a handbasket with respect to resistance

4. Then again, maybe we are…
Brief history of antimicrobials

- Antimicrobials are “magic bullets” *sensu* Ehrlich

- First modern antimicrobial was **Salvarsan**, an arsenic-based magic bullet discovered by the German infectious disease specialist Paul Ehrlich. Used to treat syphilis

- Quinine became widely used as an antimalarial after it was isolated in 1820 from the bark of the cinchona tree

- Sulfonamides were introduced in the 1930s. They are synthetic antimicrobials that block folic acid production in bacteria
Brief history of antimicrobials

- The first **antibiotic** (in the original sense of the word) was **penicillin**

- The term “antibiotic” originally was used to denote formulations derived from living organisms but is now used for partially or wholly synthetic antimicrobials too

- The French physician Ernest Duchesne first noted that certain moulds kill bacteria, but his work was forgotten

- Alexander Fleming rediscovered that *Penicillium* kills bacteria in 1928
Brief history of antimicrobials

- Fleming was convinced that the observation could never lead to therapeutic agents.
- Florey and Chain resurrected the work, isolated penicillin, and by WWII were treating millions with antibiotics.
- The age of antibiotics changed the landscape of modern medicine and antibiotics are one of the key medical interventions that have impacted human health.
Evolution of resistance

- For humans, antibiotics are lifesaving drugs; for bacteria, they are powerful agents of selection.

- When applied to a population of bacteria, an antibiotic quickly sorts out the resistant individuals from the susceptible ones.

- An evolutionary perspective suggests these drugs should be used judiciously; otherwise, these miracle drugs may undermine their own success.
Evolution of resistance

• There are dozens of antibiotics and dozens of molecular mechanisms whereby bacteria can become resistant
ANTIBIOTIC-RESISTANT BACTERIA owe their drug insensitivity to resistance genes. For example, such genes might code for “efflux” pumps that eject antibiotics from cells (a). Or the genes might give rise to enzymes that degrade the antibiotics (b) or that chemically alter—and inactivate—the drugs (c). Resistance genes can reside on the bacterial chromosome or, more typically, on small rings of DNA called plasmids. Some of the genes are inherited, some emerge through random mutations in bacterial DNA, and some are imported from other bacteria.
**Evolution of resistance**

- *Mycobacterium tuberculosis* provides an example

- Isoniazid poisons bacteria by interfering with components of the cell wall.

- Before it can do so, however, it must be converted into an active form by the gene *KatG*

- Mutations in *KatG* that reduce or eliminate its activity render bacteria tolerant to isoniazid’s effects

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*Mycobacterium tuberculosis*

Causes tuberculosis; some multidrug-resistant strains are untreatable (H/C; 1970s)
Evolution of resistance

- Other mechanisms involve gains of function
- Many extrachromosomal elements of bacteria, like plasmids and transposons, carry genes conferring resistance to one or more antibiotics
- The plasmids $Tn3$, for example, found in $E. coli$, contains a gene called $bla$
- This gene encodes an enzyme, $\beta$-lactamase, that breaks down ampicillin
BACTERIA PICK UP RESISTANCE GENES from other bacterial cells in three main ways. Often they receive whole plasmids bearing one or more such genes from a donor cell (a). Other times, a virus will pick up a resistance gene from one bacterium and inject it into a different bacterial cell (b). Alternatively, bacteria sometimes scavenge gene-bearing snippets of DNA from dead cells in their vicinity (c). Genes obtained through viruses or from dead cells persist in their new owner if they become incorporated stably into the recipient’s chromosome or into a plasmid.
Evidence that antibiotics select for resistant bacteria

- Evidence comes from a variety of studies across many scales

- On the smallest scale, William Bishai and colleagues monitored an AIDS patient with tuberculosis

- Upon diagnosis, they cultured bacteria and found them sensitive to a variety of antibiotics including rifampin

- Treated with rifampin
Evidence that antibiotics select for resistant bacteria

- Tuberculosis became undetectable
- Patient relapsed and died, with resurgence of tuberculosis
- Bacteria were resistant to rifampin, with sequencing indicating a single point mutation was to blame, and that the mutation had arisen in that patient
Evidence that antibiotics select for resistant bacteria

- On a larger scale, researchers can compare the incidence of susceptible versus resistant bacterial strains in newly diagnosed, untreated patients versus those who have relapsed after treatment.

- If antibiotics select for drug resistance, expect a higher fraction of relapsed patients with resistant bacteria.

- One study on resistance to isoniazid in tuberculosis patients showed 8.2% of new cases to carry resistant bacteria versus 21.5% of relapsed cases.
Evidence that antibiotics select for resistant bacteria

- On the largest scale, researchers can evaluate the relationship over time between the fraction of patients with resistant bacteria and the society-wide level of antibiotic use:

Frequency of penicillin resistance among *Pneumococcus* bacteria in Icelandic children as a function of time.

Austin et al. (1999)
• Data on penicillin resistance in *Pseudomonas* was plotted for kids in Iceland.
• In the late 1980s and early 1990s resistance rose dramatically
• Public health authorities campaigned against penicillin overuse starting in 1992, consumption dropped
Evaluating the costs of resistance to bacteria

- Why do you think penicillin resistance dropped?
- Why would there be costs?
- What is the prediction if there are costs?
- What if all susceptible variants are wiped out (various scales)?
- When might costs fail to persist?
Stephanie Schrag and colleagues (1997) investigated whether costs in *E. coli* of resistance to streptomycin can disappear over time.

- Screened for SM resistant mutants

- SM interferes with protein synthesis by binding to *rpsL* gene product

- Point mutations in *rpsL* can render them resistant

- In one experiment, resistant strains were restored to wild-type by splicing in a normal *rpsL* gene

- If resistance comes with a cost, what should happen when the resistant strains compete with sensitive strains in culture?
• What happens if you give resistant strains a long time to evolve, then do the same competition experiment?
The rise and fall of resistance

- Appearance and growth of antimicrobial resistance requires several steps

- The rate of spread of resistance depends on the rate at which these steps are accomplished

- First, resistance must be genetically and physiologically possible (group A streptococci have not evolved resistance to penicillin in >50 years)

- A second step required for many clinically important resistance mechanisms is transfer of genes from another bacterial species (can be rare or common)
The rise and fall of resistance

- Third, for the prevalence of resistance to spread in the host population (i.e. new hosts get resistant strains) resistant pathogen must colonize new hosts.

- The rate at which this occurs plays a key role in determining the timescale on which resistance spreads.

- For bacteria that colonize hospitalized patients, this can occur on a scale of days, or less, via transmission by healthcare workers or environmental contamination, resulting in explosive outbreaks of resistant bacteria (cf HSV, spread and generation time).
Fig. 2. Changes in the proportion of patients in an intensive care nursery infected with gentamicin- and methicillin-resistant *Staphylococcus aureus* (MRSA) in 1979, by month of discharge. Antibiotic prescribing controls, cohorting of infants colonized with the resistant strain, and infection-control measures were instigated on August 16. This figure shows the proportion of all patients infected with the resistant strain, a different measurement scale from Fig. 1, which shows the proportion of all isolates that were resistant. Modified from Ref. 42.
The rise and fall of resistance

• Finally, resistance often substantially impairs the growth rate or transmissibility of some pathogens, thereby limiting the ability of resistant infections to spread (evolutionary cost)

• Different rates of compensatory evolution will thus help determine the rise and fall of resistance

• Different pathogen/antimicrobial combinations will achieve and reverse these steps at different rates, so there is not one single pattern of resistance evolution that can be applied universally
Questions for future research

- What role does multiple resistance play in frustrating efforts to reduce resistance to individual drugs?
- How do patterns of antibiotic use contribute to (or help to slow) the appearance of such strains?
- How useful will vaccines be in combating resistance, and for how long?
- To what degree are resistant organisms actually circulating in human populations suffering from a fitness cost of resistance, compared with their susceptible counterparts?
- What role does infection control play in the prevention of drug-resistant infections in particular?
Antiviral resistance

- There are several antiviral drugs available, and as with bacteria, the selective pressure exerted by the antimicrobial can lead to resistance

- We’ll look in detail at the evolution of resistance to AZT in HIV

- First let’s look at influenza and HSV
Antiviral resistance

- A model of the use of amantadine and rimantadine during and influenza epidemic predicted that substantial levels of resistance would arise within weeks of widespread antiviral use.

- *WHY?*
Antiviral resistance

- High probability of initial emergence of resistance (30% in a treated host)
- Resistant forms are highly transmissible
- Short generation time (days)
- High efficacy = strong selection for resistance
Antiviral resistance

- Similar studies of resistance to nucleoside analogs in HSV-1 and -2 predict that it would take decades or longer for resistance to get to even a few percent.

- WHY?
Antiviral resistance

- Low probability of initial emergence of resistance (0-0.2% in a treated host)
- Resistant forms have reduced transmissibility
- Long generation time (years)
- Low efficacy = weak selection for resistance
Common Flu Drugs Meet Growing Resistance

By Neil Osterweil, Senior Associate Editor, MedPage Today
Reviewed by Rubeen K. Israni, M.D., Fellow, Renal-Electrolyte at University of Pennsylvania School of Medicine
September 21, 2005
Also covered by: MSN, MSNBC

MedPage Today Action Points

• Be aware that in the event of a global influenza pandemic, the agents amantadine and rimantadine may be ineffective at preventing influenza A infections or ameliorating their severity.

• Understand that influenza vaccines are modestly effective at protecting elderly patients in long-term care settings, but are less effective at protecting community-based elderly.

Review

ATLANTA, Sept. 21-About 12% of influenza A strains worldwide have developed resistance to the most commonly used antiflu drugs, including avian flu strains found in poultry and people in Asia.
"We were alarmed to find such a dramatic increase in drug resistance in circulating human influenza viruses in recent years," said Rick A. Bright, Ph.D., of the National Center for Infectious Diseases at the CDC here in a special online edition of The Lancet.

"Our report has broad implications for agencies and governments planning to stockpile these drugs for epidemic and pandemic strains of influenza," said Dr. Bright and colleagues at the CDC and the Wisconsin State Laboratory of Hygiene in Madison.

"With the increasing rates of resistance shown here, amantadine and rimantadine will probably no longer be effective for treatment or prophylaxis in the event of a pandemic outbreak of influenza."
Antiviral Resistance in Influenza Viruses — Implications for Management and Pandemic Response

Frederick G. Hayden, M.D.
### Incidence of M2-Inhibitor Resistance among Human Influenza A (H3N2) Viruses in the United States.*

<table>
<thead>
<tr>
<th>Period</th>
<th>No. of Isolates Tested</th>
<th>No. That Showed Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992–1995</td>
<td>991</td>
<td>8 (0.8)</td>
</tr>
<tr>
<td>1996–1997</td>
<td>508</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>1998–1999</td>
<td>510</td>
<td>11 (2.2)</td>
</tr>
<tr>
<td>2000–2001</td>
<td>283</td>
<td>4 (1.4)</td>
</tr>
<tr>
<td>2002</td>
<td>290</td>
<td>4 (1.4)</td>
</tr>
<tr>
<td>2003</td>
<td>174</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>2004</td>
<td>466</td>
<td>9 (1.9)</td>
</tr>
<tr>
<td>October 2004–March 2005</td>
<td>636</td>
<td>92 (14.5)</td>
</tr>
<tr>
<td>October–December 2005</td>
<td>209</td>
<td>193 (92.3)</td>
</tr>
</tbody>
</table>

* Data for 1992 through 1995 are from Ziegler et al. The total includes up to 20 percent influenza A (H1N1) viruses, all of which were susceptible to M2 inhibitors. All resistant viruses were influenza A (H3N2); seven of eight resistant variants contained the Ser31Asn mutation. Data for 1998 through the October 2004–March 2005 period are from Bright et al. The Ser31Asn mutation was found in 98.2 percent of the resistant variants. From 1998 through 2004, resistance was observed in 2 of 589 H1N1 viruses collected worldwide (0.3 percent; Val27Ala and Glu34Lys) and in 1 of 83 H1N2 viruses isolated in the United States (Ala30Thr). Data for October through December 2005 are from Bright et al. Six H3N2 viruses with Ser31Asn also contained Val27Iso in M2; two of eight H1N1 isolates had Ser31Asn.
Amantadine blocks the influx of H\(^+\) ions through the M2-proton channel, inhibiting uncoating and release of free ribonucleoproteins into the cytoplasm.
Mechanism of Action of and Development of Resistance to M2 Inhibitors.

In the absence of amantadine, the proton channel mediates an influx of H\(^+\) ions into the infecting virion early in the viral replication cycle, which facilitates the dissociation of the ribonucleoproteins from the virion interior and allows them to be released into the cytoplasm and transported into the cell nucleus. In highly pathogenic avian viruses (H5 and H7), the M2-proton channel protects the hemagglutinin from acid-induced inactivation in the trans-Golgi network during transport to the cell surface. In the presence of amantadine, the channel is blocked and replication is inhibited. The serine at position 31 lies partially in the protein–protein interface and partially in the channel (see inset). Replacement of serine by a larger asparagine leads to the loss of amantadine binding and the restoration of channel function. Depending on the particular amino acid, other mutations at position 26, 27, 30, or 34 may inhibit amantadine binding or allow binding without the loss of ion-channel function. Inset courtesy of Rupert Russell, Phillip Spearpoint, and Alan Hay, National Institute for Medical Research, London.
Antiviral resistance

- Why does AZT work in the short run, against HIV, but fail in the long run?

- AZT = azidothymidine

- Note the thymidine: it’s a nucleoside analogue that tricks the virus’s reverse transcriptase

- RT uses nucleotides from host cell to build a DNA strand complementary to the viral genomic RNA

- AZT mimics a normal nucleotide well enough to fool RT, but lack the attachment site for the next nucleotide in the chain
The war within the host

- Normal DNA building blocks end with hydroxyl group (-OH)
- AZT looks just like a proper building block except for the azide group
- -OH group is required for the next building block to be attached to the DNA molecule
Above is just one artist's concept of Alaska's future bridge that will connect the 8,000-resident village of Ketchikan with Gravina Island, population 50.
The war within the host

Virion susceptible to AZT
 Virion partly resistant to AZT
Virion highly resistant to AZT

Time
Antiviral resistance: HIV and AZT

Virion susceptible to AZT
Virion partly resistant to AZT
Virion highly resistant to AZT

Errors in reverse transcription generate a variable population. Some variants differ in resistance to AZT.

Resistance is passed from parents to offspring.

During treatment with AZT, many virions fail to reproduce.

The variants that persist are the ones that can reproduce in the presence of AZT.

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The war within the host

Antiviral resistance: HIV and AZT

(a) Susceptible

Resistant

Reverse Transcriptase

N3

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The war within the host

Antiviral resistance: HIV and AZT

(a) Resistance of HIV in two patients, followed over time

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Antiretroviral therapy

• Natural selection quickly breeds for resistance to AZT

• What was needed was a way to increase the number of mutations that must arise in a virion’s genome to render it resistant to the drug

• The key breakthrough was to use combination therapy, cocktails of multiple drugs acting together which are not only very effective, but which delay evolution of resistance

Figure 3 Trends in annual rates of leading causes of death among adults aged 25–44 years in USA over the period 1982–1998. The data from 1998 are preliminary. (Source: Centers for Disease Control and Prevention.)

Such cocktails have been given the nickname Highly Active Anti-Retroviral Therapy (HAART).
Antiretroviral therapy

• The impact on the natural history of AIDS in US/Western Europe has been huge

• Opportunistic disease has been reversed and prevented

• Healthcare costs have diminished

• Many ill and disabled patients have returned to normal and functional lifestyles (some with large VISA bills)

• But all this comes at a cost:

• Expense, inconvenience, toxicity, resistance. At the moment, drug therapy is still mostly for the economically privileged
Antiviral resistance

- On what time scale does resistance arise?
- What happens when AZT treatment stops?
- What could you do to reduce the chances of resistance to AZT arising?
What can we do, in general, to fight antimicrobial resistance?

Infections caused by resistant bacteria can strike anyone—the young and the old, the healthy and the chronically ill. Antibiotic resistance also is a serious problem for patients whose immune systems are compromised, such as people with HIV/AIDS and patients in critical care units.

* About 2 million people acquire bacterial infections in U.S. hospitals each year, and 90,000 die as a result. About 70 percent of those infections are resistant to at least one drug, according to the Centers for Disease Control and Prevention.

* The total cost of antimicrobial resistance to U.S. society is nearly $5 billion annually, according to the Institute of Medicine (IOM). Treating resistant pathogens often requires more expensive drugs and extended hospital stays.

* IOM and federal agencies have identified antibiotic resistance and the dearth of antibiotic R&D as increasing threats to public health.

* *Staphylococcus aureus* (staph) is a common cause of hospital infections that can spread to the heart, bones, lungs, and bloodstream with fatal results. In 2002, 57.1 percent (an estimated 102,000 cases) of the staph bacteria found in U.S. hospitals were methicillin-resistant (MRSA), according to CDC.
What can we do, in general, to fight antimicrobial resistance?

* Although MRSA used to be limited primarily to hospital patients, it is becoming increasingly common in the broader community. A study of children with community-acquired staph infections at the University of Texas found nearly 70 percent infected with MRSA. In a 2002 outbreak, 235 MRSA infections were reported among military recruits at a training facility in the southeastern United States. In addition, 12,000 cases of community-acquired MRSA were found in three correctional facilities in Georgia, California, and Texas between 2001 and 2003.

* Since 2000, CDC has reported outbreaks of MRSA among athletes, including college football players in Pennsylvania, wrestlers in Indiana, and a fencing club in Colorado. In September of 2003, this issue was brought to national attention when MRSA broke out in Florida among the Miami Dolphins, sending two players to the hospital for treatment.
What can we do, in general, to fight antimicrobial resistance?

* Vancomycin-resistant enterococci (VRE) can cause wound infections, infections in blood, the urinary tract and heart, and life-threatening infections for hospital patients. In 2002, 27.5 percent (an estimated 26,000 cases) of tested enterococci samples from ICUs were resistant to vancomycin, according to CDC.

* The percentage of *Pseudomonas aeruginosa* bacteria resistant to either ciprofloxacin or ofloxacin, two common antibiotics of the fluoroquinolone class (FQRP), has increased dramatically. Recent CDC data show that in 2002, nearly 33 percent of tested samples from ICUs were resistant to fluoroquinolones. *P. aeruginosa* causes infections of the urinary tract, lungs, and wounds and other infections commonly found in intensive care units.
Some Actions Physicians and Consumers Can Take to Limit Resistance

The easy accessibility to antibiotics parodied in the cartoon is a big contributor to antibiotic resistance. This list suggests some immediate steps that can help control the problem.

—S.B.L.

Physicians

• Wash hands thoroughly between patient visits.
• Do not accede to patients' demands for unneeded antibiotics.
• When possible, prescribe antibiotics that target only a narrow range of bacteria.
• Isolate hospital patients with multidrug-resistant infections.
• Familiarize yourself with local data on antibiotic resistance.

Consumers

• Do not demand antibiotics.
• When given antibiotics, take them exactly as prescribed and complete the full course of treatment; do not hoard pills for later use.
• Wash fruits and vegetables thoroughly; avoid raw eggs and undercooked meat, especially in ground form.
• Use soaps and other products with antibacterial chemicals only when protecting a sick person whose defenses are weakened.

"Don't forget to take a handful of our complimentary antibiotics on your way out."
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What can we do, in general, to fight antimicrobial resistance?

- Economic changes
- Regulatory changes
- Scientific changes

Antibiotics at the crossroads

Are we making the right choices to bring new drugs to the marketplace?

Oct 21 2004 issue of Nature
What can we do, in general, to fight antimicrobial resistance?

Economic changes

• Demand for blockbusters for chronic disease

• Broad spectrum antibiotics = wider market

• Pressure to spare use as resistance increases = bad investment

• Profits restrained in medical arena, pharma sends about half of output to food industry