Evolution of antibiotic resistance

October 10, 2005
Causes of death, 2001:

1. Infectious and parasitic diseases: 14.9 million
2. Heart diseases: 11.1 million
3. Cancers: 7.3 million
4. Stroke: 5.5 million
5. Respiratory diseases: 3.6 million
6. Accidents, fires, drowning, etc.: 3.5 million
7. Maternal and perinatal: 3.0 million
8. Violence (war, homicide, suicide): 1.6 million

Population: 6,122,210,000
Deaths: 56,554,000

World Health Organization
World Health Report 2002
Infectious diseases in the news

3 October 2005

Australians Win Nobel Medicine Prize for Ulcer Breakthrough
Voice of America - 23 minutes ago
Two Australian researchers have won the Nobel Prize for Medicine for their discovery that stomach and intestinal ulcers are caused by bacteria, not stress.
Discovery of Ulcer's Germ Etiology Wins Medicine Nobel

WHO backs away from 150 million flu deaths
Reuters - Sep 30, 2005
The World Health Organisation (WHO) said on Friday 2-7.4 million deaths was a reasonable working forecast for a global influenza pandemic -- distancing itself ...

Some Dole salads connected to E. coli
San Jose Mercury News - 5 hours ago
WASHINGTON - The Food and Drug Administration is warning people not to eat certain Dole pre-packaged salads that have been connected to an outbreak of E. coli infections in Minnesota.
Warning about some Dole salads
E. coli outbreak triggers nationwide alert over packaged salad mix

FDA.gov - Food Consumer - KDKA - WGRZ-TV - all 103 related »
Deaths from infectious diseases in the US: 1900-1994

1900-1937: public health, clean water, good sewers
1937-1953: vaccines, antibiotics
1953-1980: antibiotics, antivirals
1980-1994: still more drugs, but...

1918 flu epidemic
Deaths from infectious diseases in the US: 1900-1996
FROM ORDINARY MOLD—
the Greatest Healing Agent of this War!

When the dangerous bacteria of this war have invaded the body, they are destroyed by penicillin. Penicillin is a mold that grows in many different places, and when it is ground up, it is useful in treating many diseases. The discovery of penicillin has helped to save many lives and has increased the chances of recovery for patients suffering from various illnesses.

Keep this information in mind and use penicillin whenever possible. It is a valuable tool in the fight against disease.

A great many patients have received treatment with penicillin, and they have been able to recover more quickly. It is available in various forms, including pills and ointments.

Clover to THE DECISION HOUSE, 547-1328. Buried in the soil, penicillin is produced by Penicillin.
## Antibiotic discovery and resistance development

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Discovered</th>
<th>First clinical use</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>1940</td>
<td>1943</td>
<td>1940</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1944</td>
<td>1947</td>
<td>1947</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1948</td>
<td>1952</td>
<td>1956</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1952</td>
<td>1955</td>
<td>1956</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1956</td>
<td>1972</td>
<td>1987</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1963</td>
<td>1967</td>
<td>1970</td>
</tr>
</tbody>
</table>
Penicillin

β-lactams

Lysis of E. coli by Penicillin
© James A. Sullivan
Quill Graphics
Charlottesville, VA USA
Bacterial cell surface structure

- Teichoic acid
- Peptidoglycan layer (cell wall)
- Outer membrane
- Periplasmic space
- Inner membrane
- Membrane proteins
- Lipopolysaccharide (LPS) outer leaflet of outer membrane
- Pore protein
- Peptidoglycan

Gram Positive

Gram Negative
Cell wall molecular structure
Transpeptidase reaction and penicillin inhibition
Antibiotic targets: mostly cell wall and ribosome

Modes of antibiotic resistance

- Destroy or covalently modify the drug
- Change the target so the drug no longer binds
- Actively export the drug from the cytoplasm by a specific or non-specific efflux pump (MDR = multi-drug resistant)
- Prevent drug uptake by altering membrane permeability (rare)

Selective pressures caused by human misuse:

- Physician overprescription
- Agricultural use as a growth enhancer
- Domestic misuse (compare the “hygiene hypothesis”)

[Image of two soap bottles]
Penicillin resistance

• Alteration in the transpeptidase (PBP)
  – Usually generates cross-resistance to all β-lactams
  – Mechanism found in MRSA (mecA gene acquired laterally from unknown source)

• Expression of β-lactamases
  – At least 255 different kinds
  – Derived from transpeptidases!!!
  – Rate of hydrolytic deacylation increased from 1 per hour to 1500 per second
  – Can be partially overcome by coadministration of clavulanic acid (augmentin)
Nosocomial infections

- >10 per 1000 patient-days in the hospital
- Most common in intensive care units, acute care surgical and orthopedic units
- Increasing in frequency and severity
  - Populations are more immunocompromised
  - Antibiotic resistance is becoming more prevalent
- Frequently opportunistic Gram-positives from normal flora (Staphylococcus, Enterococcus, Streptococcus)
- MRSA (methicillin-resistant Staphylococcus aureus) are often resistant to all antibiotics except vancomycin
- MRSA increasingly found in community-acquired infections as well as hospital-acquired infections
  - Two clinical papers for Friday
## PENICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS*

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Data Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940</td>
<td>Virtually all strains susceptible to penicillin (worldwide)</td>
<td></td>
</tr>
<tr>
<td>1940-1946</td>
<td>(Finland, BCH)</td>
<td>&lt;1% Resistant</td>
</tr>
<tr>
<td>1947</td>
<td>(Finland, BCH)</td>
<td>32% Resistant</td>
</tr>
<tr>
<td>1951</td>
<td>(Finland, BCH)</td>
<td>73% Resistant</td>
</tr>
<tr>
<td>1967</td>
<td>(Moellering, MGH)</td>
<td>83% Outpatient isolates resistant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84% Inpatient isolates resistant</td>
</tr>
</tbody>
</table>

Currently – 90% Resistant worldwide

METHICILLIN-RESISTANT S. AUREUS
Methicillin resists most β-lactamases

HISTORICAL ASPECTS

1959  First clinical use of methicillin
1961  First description of MRSA
1967  First report of nosocomial infection in the US (2 cases)
1968  Increase in MRSA in the UK
1968-1979 Rise and subsequent wane of prevalence of MRSA (especially nosocomial infections) in Europe, Australia, and elsewhere (except US)
1975-1980 First reports of problems with MRSA in the US; most occurred in large tertiary care hospitals (especially burn units and ICUs)
1980-1991 MRSA increase in prevalence in US nursing homes; community-acquired MRSA infections in the US

1Jevons. BMJ 1961;1:124
Figure 2. International spread of the pandemic MRSA clones.

Oliveira et al., 2002, Lancet Inf Dis. 2: 180
Vancomycin

20-50% of a typical hospital antibiotic budget is spent on vancomycin.
Vancomycin resistance in enterococci

- 12 species cause bacteremia, mostly *E. faecalis* and *E. faecium*
- Vancomycin resistance described in 1986; currently 25% of clinical isolates are resistant (VRE)
- High mortality rate (10-50%)
- US: Reservoirs are hospital staff and patients (farm animals in Europe due to use of avoparcin)
- Genotypic classification of resistance:
  - vanA - inducible, cross resistance to teicoplanin, >1000 µg/ml
  - vanB - inducible, teicoplanin-sensitive, >1000 µg/ml
  - vanC, vanD - constitutive, teicoplanin-sensitive, 30-100 µg/ml
vanA: Organization of transposon Tn1546

- orf1 - transposase
- orf2 - resolvase
- vanR - response regulator (transcriptional activator)
- vanS - histidine protein kinase (sensor)
- vanH - D-specific α-keto acid reductase (makes D-lactate)
- vanA - D-Ala-D-lactate peptide ligase
- vanX - D-Ala-D-Ala dipeptidase
- vanY - D-D carboxypeptidase
Induction of resistance genes by vancomycin via two-component response regulator
Change of cell wall peptide from D-Ala-D-Ala to D-Ala-D-lactate removes one hydrogen bond...enough!

\[
\begin{align*}
    \text{CH}_3 & \\
    \text{H}_2\text{N} & \text{C} \text{COOH} \\
    \text{H} & \\
    \text{alanine} & \\
    \text{CH}_3 & \\
    \text{HO} & \text{C} \text{COOH} \\
    \text{H} & \\
    \text{lactate} &
\end{align*}
\]
Mechanisms of genetic exchange and spread of resistance determinants

Known cross-species routes of exchange
VRE can transfer Tn1546 to MRSA in vitro
(samples immediately autoclaved)
Well, has transfer occurred?

- June 2002: 40 yo woman in Michigan
  - Hypertension, diabetes, peripheral vascular disease, chronic renal failure
  - Recurrent foot ulcers due to diabetic neuropathy; right foot amputated
  - Treated with vancomycin, gentamicin, ampicillin-sulbactam, piperacillin-tazobactam, levofloxacin, clindamycin, cefazolin, trimethoprim-sulfamethoxazole, tobramycin and metronicazole prior to amputation
  - Cultured MRSA in April 2002, VRE in June 2002
  - VRSA appeared in June 2002: Tn1546 transferred from VRE on a conjugative plasmid (pLW1043)

Chang et al., 2003, NEJM 348: 1342
Weigel et al., 2003, Science 302: 1569
Other paths to resistance: genomic mutation, particularly important for quinolones

**Ciprofloxacin**
- Inhibits DNA Topoisomerase
- Point mutations in GyrA give resistance

**A-692345**
- Inhibits protein synthesis
  - (S. pneumoniae, H. influenzae)

Dandliker, et. al. AAC (2003), 47, 3831.
Bacterial stress response: SOS

 lexA discovered by Paul Howard-Flanders and Lee Theriot in 1966
Screen for mutants involved in DNA repair after UV or X-ray treatment and in DNA recombination
lexA required for repair but not recombination

PAPER FOR FRIDAY:
GyrA inhibition by Cipro induces SOS response and mutagenesis (shades of Lamarck)