Combating antibiotic resistance

October 23, 2006
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
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
Thanks to PENICILLIN
...He Will Come Home!

FROM ORDINARY MOLD—
the Greatest Howling
Agent of this War!

When the thousands of bottles of this revolutionary drug are opened in a laboratory, the germ-killing effect of the War Austen will be the discovery and development of the amazing new weapon that destroys...not a weapon that serves here. That weapon, nature, is penicillin.

Every day, penicillin is performing, in medical and scientific work, a greater service than ever imagined. In the future, penicillin may be used in other ways that we cannot foresee. It is available to all men everywhere.
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>
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<tr>
<td>Streptomycin</td>
<td>1944</td>
<td>1947</td>
<td>1947</td>
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<tr>
<td>Tetracycline</td>
<td>1948</td>
<td>1952</td>
<td>1956</td>
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<tr>
<td>Erythromycin</td>
<td>1952</td>
<td>1955</td>
<td>1956</td>
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<tr>
<td>Vancomycin</td>
<td>1956</td>
<td>1972</td>
<td>1987</td>
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<tr>
<td>Gentamicin</td>
<td>1963</td>
<td>1967</td>
<td>1970</td>
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</tbody>
</table>
Penicillin

β-lactams
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 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
METHICILLIN-RESISTANT S. AUREUS

*Methicillin resists most β-lactamases*

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1959</td>
<td>First clinical use of methicillin</td>
</tr>
<tr>
<td>1961</td>
<td>First description of MRSA</td>
</tr>
<tr>
<td>1967</td>
<td>First report of nosocomial infection in the US (2 cases)</td>
</tr>
<tr>
<td>1968</td>
<td>Increase in MRSA in the UK</td>
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<tr>
<td>1968-1979</td>
<td>Rise and subsequent wane of prevalence of MRSA (especially nosocomial infections) in Europe, Australia, and elsewhere (except US)</td>
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<tr>
<td>1975-1980</td>
<td>First reports of problems with MRSA in the US; most occurred in large tertiary care hospitals (especially burn units and ICUs)</td>
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<td>1980-1991</td>
<td>MRSA increase in prevalence in US nursing homes; community-acquired MRSA infections in the US</td>
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<tr>
<td>2003-2004</td>
<td>Community-acquired clones of MRSA cause outbreaks of necrotizing fasciitis in Los Angeles</td>
</tr>
</tbody>
</table>

Pandemic MRSA around the world

Figure 2. International spread of the pandemic MRSA clones.

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

\[
\begin{align*}
\text{alanine} & : \quad \text{CH}_3 \\
\text{H}_2\text{N} & - \text{C} - \text{COOH} \\
\text{H} & \\
\text{lactate} & : \quad \text{HO} - \text{C} - \text{COOH} \\
\text{H} & 
\end{align*}
\]
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
Isn’t there a fitness cost?

Clinical isolates of rifampicin-resistant *Mycobacterium tuberculosis* have little or no fitness defect; laboratory isolates always do

Gagneux et al., 2006, *Science* 312: 1944

Also: bacteria under antibiotic stress
1) increase genetic transformability
2) increase error-prone replication mechanisms
Pseudomembranous colitis:
a disease caused by antibiotics

3% of healthy adults and 20-40% of hospitalized patients are colonized with spores of *Clostridium difficile*

Killing normal flora with antibiotics allows *C. difficile* outgrowth (clindamycin, cephalosporins, fluoroquinolones all implicated)

Two toxins cause cell death and inflammation

“Pseudomembrane” is made of fibrin, inflammatory cells, necrotic tissue, overlying the mucosa

PAPER 1: McDonald et al. New, more virulent strains are spreading
What can we do? New targets, new drugs…

**Ciprofloxacin**

Inhibits DNA Topoisomerase

Point mutations in GyrA give resistance

**A-692345**

Inhibits protein synthesis

*(S. pneumoniae, H. influenzae)*

ClpP protease is involved in regulated degradation of cytoplasmic proteins (compare the eukaryotic proteasome).

ΔclpP mutants are viable.

ClpP normally requires an ATPase partner (e.g. ClpA) to activate proteolysis.

PAPER 2: Brotz-Oesterhelt et al. What happens if it is misregulated?