Combating antibiotic resistance

October 8, 2007

Causes of death, 2001:

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

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

World Health Organization
World Health Report 2002
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...

Deaths from infectious diseases in the US: 1900-1996

1980-1994
Resistance to Antibiotics Is Inevitable and Develops Rapidly

- sulfonamides
- penicillin G
- streptomycin
- tetracycline
- erythromycin
- nalidixic acid
- vancomycin
- methicillin
- expanded-spectrum cephalosporins
- Augmentin
- norfloxacin
- linezolid

Timeline:

- 1930
- 1940
- 1950
- 1960
- 1970
- 1980
- 1990
- 2000
Penicillin

1928: Mold found by Alexander Fleming (who also found lysozyme by sneezing on a plate)
1940: Penicillin purified by Florey & Chain

β-lactams

Bacterial cell surface structure

[Diagram of bacterial cell surface structure showing teichoic acid, peptidoglycan layer, outer membrane, periplasmic space, inner membrane, cytosol, GRAM POSITIVE, GRAM NEGATIVE, lipopolysaccharide (LPS) outer leaflet of outer membrane, pore protein, peptidoglycan, membrane proteins.]
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”)
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
## PENICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS

Currently – 90% Resistant worldwide

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Type</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940</td>
<td>Worldwide</td>
<td>Strains</td>
<td>Virtually all susceptible</td>
</tr>
<tr>
<td>1940-1946</td>
<td>Finland, BCH</td>
<td>outpatient isolates</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>1947</td>
<td>Finland, BCH</td>
<td>hospitalized patients (outpatient isolates susceptible)</td>
<td>32%</td>
</tr>
<tr>
<td>1951</td>
<td>Finland, BCH</td>
<td>hospitalized patients (inpatient isolates susceptible)</td>
<td>73%</td>
</tr>
<tr>
<td>1967</td>
<td>Moellering, MGH</td>
<td>outpatient isolates</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>inpatient isolates</td>
<td>84%</td>
</tr>
</tbody>
</table>


## 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>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>1980-1991</td>
<td>MRSA increase in prevalence in US nursing homes; community-acquired MRSA infections in the US</td>
</tr>
<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

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!

Vancomycin binds to D-Ala-D-Ala-termini (5 hydrogen bonds)
Synercid for treating VRE (E. faecium only)

Bind 50S ribosomal subunit
Bacteriostatic alone
Bacteriocidal together
Advertised as a “new class”

BUT...

Comparison of % resistance among Enterococcus faecium from food animals, food and humans (1997, Denmark).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Chick</th>
<th>Cattle</th>
<th>Beef</th>
<th>Pigs</th>
<th>Pork</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>6</td>
<td>0</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>9</td>
<td>20</td>
<td>36</td>
<td>87</td>
<td>26</td>
<td>8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>71</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Penicillin</td>
<td>2</td>
<td>13</td>
<td>3</td>
<td>47</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>59</td>
<td>13</td>
<td>3</td>
<td>37</td>
<td>10</td>
<td>29</td>
</tr>
</tbody>
</table>

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

Growth curves for a vancomycin-dependent isolate of VRE

1992
46 yo woman
Infected with S. aureus, Pseudomonas aeruginosa, Enterobacter spp.
Two 6-10 week courses of vancomycin (plus others)
Urine samples plated as sterile but chains of Gram-positive cocci observed in the microscope
What happened? (Hint: loss of gene)
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

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*)

Inhibiting pathogenesis as a novel therapeutic strategy

Phenotypic screen in *Vibrio cholerae* identified “virstatin” (4-[N-(1,8-naphthalimide)]-n-butyric acid)
NO effect on growth, inhibits expression of cholera toxin and TCP
Directly inhibits ToxT transcription factor
Selective pressures???

Hung et al., 2005, Science 310: 670

Papers for Wednesday: