A Race Against Resistance

Deborah T. Hung, M.D.-Ph.D.

Broad Institute of MIT and Harvard
and
Department of Molecular Biology
Center for Computational and Integrative Biology
Massachusetts General Hospital
and
Department of Microbiology and Molecular Genetics
Harvard Medical School

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The global problem

~ 56 million deaths/year worldwide

- Infectious diseases: 27%
- Cardiovascular disease: 30%
- Cancer: 13%
- Injuries: 10%
- Respiratory disease: 6%
- Other: 14%

worldwide
The global problem

~ 56 million deaths/year worldwide

worldwide developing nations

~ 1/3 of total deaths worldwide
Leading infectious killers

The majority are curable
> 51% of deaths in children worldwide = infection

The death of a child

Percentage of women aged 15 to 49, married or previously married, who have had at least one child die

<table>
<thead>
<tr>
<th>Country</th>
<th>Death Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkina Faso</td>
<td>30%</td>
</tr>
<tr>
<td>Columbia</td>
<td>13%</td>
</tr>
<tr>
<td>Egypt</td>
<td>32%</td>
</tr>
<tr>
<td>Indonesia</td>
<td>20%</td>
</tr>
<tr>
<td>Kenya</td>
<td>49%</td>
</tr>
<tr>
<td>Malawi</td>
<td>52%</td>
</tr>
<tr>
<td>Namibia</td>
<td>26%</td>
</tr>
<tr>
<td>Pakistan</td>
<td>30%</td>
</tr>
<tr>
<td>Peru</td>
<td>25%</td>
</tr>
<tr>
<td>Philippines</td>
<td>19%</td>
</tr>
<tr>
<td>Senegal</td>
<td>45%</td>
</tr>
<tr>
<td>United Republic of Tanzania</td>
<td>40%</td>
</tr>
<tr>
<td>Yemen</td>
<td>42%</td>
</tr>
</tbody>
</table>

Source: Demographic and Health Survey 2014
The Economic Burden

Economic burden

When infectious diseases are not controlled, they can place a tremendous burden on economies.

Economic savings

The cost of controlling or eliminating infectious diseases is often recovered many times over in future savings.

Source: WHO
Needed interventions

**Preventable deaths**
It is estimated that the majority of deaths from infectious diseases can be prevented with existing, cost-effective strategies.

**Childhood vaccinations** have proven extremely effective in reducing deaths from measles and other preventable diseases.

**Bednets** and other prevention and treatment strategies can prevent 50% of all **malaria** deaths.

**DOTS** (Directly Observed Treatment, Short-course) can prevent 60% of all **tuberculosis** deaths.

**IMCI** (Integrated Management of Childhood Illnesses) can prevent most childhood deaths from pneumonia, diarrhoea, malaria and measles. An important part of IMCI is oral rehydration therapy, which can prevent up to 90% of deaths from **diarrhoeal** diseases.

**Antibiotics** used in timely and correct doses, combined with other strategies such as IMCI, are highly effective in preventing deaths from pneumonia.

**HIV prevention strategies** such as condom promotion, sex education and treatment of STIs have been proven to reduce the spread of HIV/AIDS.

Source: WHO
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Source: WHO
Antibiotics have saved millions of lives and eased the suffering of patients of all ages for more than 60 years. These “wonder drugs” deserve much of the credit for the dramatic increase in life expectancy in the United States and around the world in the 20th century.

1928 - Sir Alexander Fleming’s discovery of penicillin
Antibiotics have saved millions of lives and eased the suffering of patients of all ages for more than 60 years. These “wonder drugs” deserve much of the credit for the dramatic increase in life expectancy in the United States and around the world in the 20th century.

“(it is) time to close the book on infectious disease.”

Time of “Crisis” and “Emergency”
# New pathogens identified since 1977

<table>
<thead>
<tr>
<th>Year</th>
<th>Pathogen(s)</th>
<th>Year</th>
<th>Pathogen(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<td>HTLV-1</td>
<td>1990</td>
<td>ESBL Gram negatives</td>
</tr>
<tr>
<td>1982</td>
<td>HTLV-2, E.coli 0157:H7, Borrelia burgdorferi</td>
<td>1993</td>
<td>Hanta virus</td>
</tr>
<tr>
<td>1983</td>
<td>HIV</td>
<td>1995</td>
<td>HHV-8</td>
</tr>
<tr>
<td>1988</td>
<td>Hepatitis E, Helicobacter pylori</td>
<td>1997</td>
<td>H5N1 - avian influenza</td>
</tr>
<tr>
<td>1988</td>
<td>HHV-6</td>
<td>2000</td>
<td>Chlamydia - CAD, West Nile virus</td>
</tr>
<tr>
<td>1990</td>
<td>ESBL Gram negatives</td>
<td>2003</td>
<td>SARS</td>
</tr>
</tbody>
</table>
Unexpected outbreaks

Examples of emerging and re-emerging infectious diseases 1994-1999

- Anthrax
- Brucellosis
- Cholera
- Crimean-Congo haemorrhagic fever
- Cryptosporidiosis
- Dengue haemorrhagic fever
- Diphtheria
- Ebola haemorrhagic fever
- E.coli O157
- Echinococcosis
- Enterovirus 71
- Epidemic meningitis
- Hendra
- Human monkeypox
- Influenza A (H5N1)
- Influenza A (H9N2)
- Lassa fever
- Leptospirosis
- Lyme borreliosis
- Malaria
- New variant CJD
- Nipah
- Omek haemorrhagic fever
- O’nyong-nyong fever
- Plague
- Poliomyelitis
- Reston virus
- Rift Valley fever
- Ross River virus
- Typhoid
- Venezuelan equine encephalitis
- West Nile fever
- Yellow fever

Source: WHO
Timeline of antibiotic deployment and evolution of antibiotic resistance
Increasing resistance among pathogens

Malaria
Quinine and mefloquine in Thailand

Note: There is already complete resistance to chloroquine and sulfadoxine-pyrimethamine in Thailand

45% resistance

Tuberculosis
Short-course chemotherapy in Portugal

13% single drug resistance

Staphylococcus in Japan

4% multidrug resistance

60% multidrug resistance

Now also XDR-TB

The US is not immune

- 2 million people - acquire bacterial infection in US hospitals/year.
- 90,000 people - die of this infection
- 70% of these infections from bacteria resistant to at least one drug
- $ 5 billion - annual cost of “resistance” (more expensive drugs, longer hospital stay)
The typical story of resistance

- 1940s  Penicillin kept Staphylococcus bacteria under control.
- 1942   Penicillin-resistant Staph bacteria were identified
- late 1960s > 80 percent of Staph bacteria were penicillin-resistant.
- 1961   Methicillin was introduced to combat resistant Staph bacteria.
- 1974   2 percent of the Staph bacteria found in U.S. hospitals methicillin-resistant.
- 2002   Jump to 57.1 percent Staph bacteria now resistant.
- 1958   Vancomycin was introduced to combat resistant Staph bacteria.
- late 1990s Staph bacteria partially resistant to vancomycin.
- 2002   Two cases of fully vancomycin-resistant Staph (VRSA).
- 2002   Recognition of community-acquired Staph infections. 70% of children at the University of Texas infected with MRSA.
Infectious diseases physicians are alarmed by the prospect that effective antibiotics may not be available to treat seriously ill patients in the near future. There simply aren’t enough new drugs in the pharmaceutical pipeline to keep pace with drug-resistant bacterial infections, so-called ‘superbugs.”

Joseph R. Dalovisio, MD
IDSA President
Can we always count on the next antibiotic? NO!

- Only 2 new antibiotics with new mechanism of action, no cross resistance in 40 years!

- 1998-2008 only 10 new antibiotics FDA approved

- In 2002, among 89 medicines emerging on market, none was an antibiotic

- In 2004, of 506 drugs in development pipeline, only 5 are antibiotics (vs. 67 in cancer)
Challenges unique to antibiotic discovery & development

- Short courses
- Limited utility when resistance develops
  (bacteria evolved much more rapidly)
- Technically difficult to find
- Goal broad spectrum antibiotics
- Limited willingness of specialists to use new drugs
- Median time for clinical testing and FDA approval 6 years
  (additional ~ 8 prior to clinical testing)
- Only 1/5000 in clinical testing make it to FDA approval
Dogma of biology: DNA to RNA to protein
Phenotype = in vitro death
Mechanisms of antibiotic resistance

- Horizontal gene transfer
- Efflux
- Degradation
- Modification
- Mutation
- Chromosome-based
Current approaches to antibiotic discovery: HTS

In vitro screening: 100K - millions

Chemical libraries for screening:

“drug-like”

valium
diversity-oriented synthesis

natural products
ginkgolide B
• Target based

• Whole cell

HTS assays for antibiotic discovery
HTS assays for antibiotic discovery
HTS assays for antibiotic discovery

...if we need to know the target?
Finding a needle in a haystack:
Generate resistant mutants and total genome sequencing to find mutation
In vitro screening for death

GSK experience: 1995-2001

70 chemical HTS performed (~ $1 million/screen)
screen 260-530K compounds

Target 300 genes

3 Whole cell screens

No real viable candidates

We have to start thinking outside of the box
What do we really care about?
What do we really care about?

cholera

hospital acquired infections

TB
Host-pathogen interactions

What we really care about - infection
A complex dance

- Encounter
- Entry
- Spread
- Multiply
- Damage
- Be transmitted

All extremely tightly regulated
Can we simply tip the balance in favor of the host?
Can we simply tip the balance in favor of the host?

1. Anti-virulence
2. In vivo essentials
3. Host
4. Anti-resistance
Host-pathogen interaction

How can we intervene to disrupt this interaction?

1. Motility/chemotaxis
2. Adherence
3. Sensing when to turn on virulence
4. Virulence regulation
5. Toxin delivery
6. Toxin function
7. Essential in vivo functions
8. Host response
Can we simply tip the balance in favor of the host?

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Inhibition of virulence: Can we disarm the bugs?

- Oral ingestion
- Passage through the gastric acid barrier
- Colonization of intestinal epithelium
- Multiplication
- Persistence in aquatic reservoirs
- Cholera toxin (TCP)
- Diarrhea

Biofilm
Inhibition of virulence: Mouse model for cholera

1. Mix bugs with drug

2. Inoculate orogastrically into infant mice

3. After 18 hours, collect small intestine, homogenize and plate onto LB streptomycin

4. Count numbers of recovered Vibrios
Inhibition of virulence: Mouse model for cholera

A new paradigm for antivirulence drugs
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Host-pathogen models for HTS
Host-pathogen models for HTS

Caenorhabditis elegans

Danio rerio
Screen of infected worms

Fred Ausubel and Terry Moy, MGH

Enterococcus faecalis
Screen of infected zebrafish embryos: transparent

Embryo infected with heat-killed GFP-tagged PA14, 24 hpi

*Pseudomonas aeruginosa*
Can we simply tip the balance in favor of the host?

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The human immune system is pretty amazing

1/3 of the world’s population is infected with TB
90% of TB infections are completely asymptomatic
Human immune cells (macrophage) are main line of defense

Can we exploit them to manage infection?
TB drugs that modulate the host immune response?

blue = human cells
green = tuberculosis bacteria
High throughput microscopic imaging

Put bacteria and cells in individual wells of multi-well plates

Add chemicals, each chemical in a different well

Automated microscope

100,000+ images, each with 500-1000 cells

Identify the small molecule that prevents TB replication in human cells
Can we simply tip the balance in favor of the host?

1. Anti-virulence
2. In vivo essentials
3. Host
4. Anti-resistance
Can we prevent resistance development?

A. Genetic resistance
   - Add antibiotics
   - Wait
   - Reculture

Genotypic resistance

B. Induced mutations leading to resistance
   - Add antibiotics
   - Reculture
   - Wait
   - Add antibiotics
   - Reculture

C. Pre-existing drug "tolerance"
   - Add antibiotics
   - Reculture
   - Wait

Phenotypic resistance

D. Induced mutations in drug tolerant cells leading to resistance
   - Add antibiotics
   - Reculture
   - Wait
   - Add antibiotics
   - Reculture

E. Inhibition of induced mutations prevents resistance
Can we prevent resistance development?

A. Genetic resistance

B. Induced mutations leading to resistance

C. Pre-existing drug "tolerance"

D. Induced mutations in drug tolerant cells leading to resistance

E. Inhibition of induced mutations prevents resistance
Bacterial stress results in hypermutation

The bacterial SOS response

*Inhibition of LexA prevents the generation of ciprofloxacin resistance in a mouse model*

Can we simply tip the balance in favor of the host?

1. Anti-virulence
2. In vivo essentials
3. Host
4. Anti-resistance
Six months after using antiretroviral therapy
Anthrax
Dr. Erik Hett
Kevin Mark
Louise Slater

Pseudomonas aeruginosa
Niklesh Chand
Dr. Anne Clatworthy
Admire Kuchena
Jenny Lee

Vibrio cholerae
Kathryn Levasseur
Desiree Yang

Mycobacterium tuberculosis
Dr. Amy Barczak
Dr. Jim Gomez
Dr. Sarah Grant
Dr. Ben Kaufmann
Dr. Motohisa Shimizu
Dr. Sarah Stanley
Dr. Yajie Wang
Carl Wivagg
John Aquadro
Peter Kim

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