What is Antimicrobial Resistance?
How Does It Develop?
Where Does It Come From?

Randall Singer, DVM, MPVM, PhD
Overview

What is resistance?
- There must be a definition

How does resistance develop?
- Treating with high doses for short periods must be better

Where does resistance come from?
- It must be due to antibiotic use

When will the resistance go away?
- Because we have begun removing antibiotics from use, it must be soon
Clinically defined as the inability to treat a specific bacterial infection with an antibiotic

- Phenotypic traits of antibiotic resistance
- Focus on factors associated with isolates possessing a Minimum Inhibitory Concentration (MIC) above a specific “resistant” threshold
The purpose of *in vitro* antimicrobial susceptibility testing is to determine how bacteria MAY respond to an antimicrobial agent *in vivo*...

- A susceptible classification implies a high probability of a favorable outcome.
- A resistant classification is a good predictive value for a poor outcome.
Epidemiological cut-off values

- Important for early detection
- Trend for “resistance”
- Lack of agreement on how to define

Problems with epidemiological cut-off values

- Decreased susceptibility ≠ clinical resistance
- Lack of harmonization of cut-offs across countries / regions and time
- May have NO predictive power
Genotypically defined as the presence of a resistance gene within the bacterium that is capable of rendering an antibiotic useless

- Prevalence and distribution of bacteria that possess a certain gene that confers resistance to a given antibiotic
- Focus on factors that affect spread of resistance genes
Antibiotics

- Low molecular-weight compounds that kill or inhibit the growth of microorganisms
- Many antibiotics are naturally produced by bacteria or fungi

What is the role of antibiotic production in nature?
- Germ warfare theory
- Levels are low, almost undetectable
- Signaling molecules?
The Nature of Resistance

- Intrinsic

- Acquired
  - Mutation
    - Fluoroquinolone resistance in *Campylobacter*
  - Gene acquisition
    - Plasmids with gene for third-generation cephalosporin resistance
    - Multi-drug resistance
The Nature of Resistance

Clonal Transmission

Horizontal Gene Transfer

- Plasmids
- Integrons
- Transposons
In 1913, Ehrlich publishes “Chemotherapeutics: Scientific Principles, Methods and Results” in Lancet. He states that “treatment should hit hard and early.” This idea was meant for tuberculosis infections. Resistance in *Mycobacterium tuberculosis* is often mediated by a single point mutation. The goal of treatment is to prevent subpopulations of mutant bacilli (the resistant ones) from emerging.
Antibiotic Use and Resistance

Lipsitch and Samore, Emerg Inf Dis, 2002
How does this relate to other situations?

- Vancomycin-resistant *Enterococcus sp.*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Both of these are based on the acquisition of genes
- These resistances are unlikely to occur *de novo* in a single person or animal following treatment
- Also important to recognize that these organisms are NOT obligate pathogens
Antibiotic Use and Resistance

Lipsitch and Samore, Emerg Inf Dis, 2002
Which is worse for resistance (and health): long-term low dose or short-term high dose?

Dogma: high doses given over short term are best

High doses may select strongly for resistance - advantage to resistant populations

Low doses for growth and disease prevention may not alter the normal bacterial flora in the host – do not kill the susceptible population
Is Resistance New?

Antibiotic Resistance Is Prevalent in an Isolated Cave Microbiome

Bhullar el al., PLoS ONE (2012)
Metagenomics

Is there a better way to predict the emergence of resistance?

Handelsman, MMBR (2004)
Is Resistance New?

Antibiotic resistance is ancient

Vanessa M. D’Costa¹,²*, Christine E. King³,⁴*, Lindsay Kalan¹,², Mariya Morar¹,², Wilson W. L. Sung⁴, Carsten Schwarz³, Duane Froese⁵, Grant Zazula⁶, Fabrice Calmels⁵, Regis Debruyne⁷, G. Brian Golding⁴, Hendrik N. Poinar¹,³,⁴ & Gerard D. Wright¹,²

Figure 1 | Stratigraphic profile and location of Bear Creek site. Elevation is given in metres above base of exposure. Permafrost samples from below Dawson tephra were dated to about 30 kyr BP. Preservation of the ice below and above the sample indicates that the sediments have not thawed since deposition. Silhouettes represent mammals and birds identified from ancient DNA sequences that are typical of the regional Late Pleistocene environment. aDNA, ancient DNA.
When will resistance go away?

Ciprofloxacin Resistance

Figure 19. Percent of *Campylobacter jejuni* Isolates from Humans, Chicken Breasts, and Chickens Resistant to Ciprofloxacin, by Year, 1997-2010

Data for ground turkey are not included due to the small number of *C. jejuni* isolates from this source. Table 46 contains resistance data for *C. jejuni* isolates from each source, by year.
Enhanced \textit{in vivo} fitness of fluoroquinolone-resistant \textit{Campylobacter jejuni} in the absence of antibiotic selection pressure

Naidan Luo*, Sonia Pereira†, Orhan Sahin*‡, Jun Lin†, Shouxiong Huang*, Linda Michel*, and Qijing Zhang**†

\textit{Campylobacter jejuni}, a major foodborne human pathogen, has become increasingly resistant to fluoroquinolone (FQ) antimicrobials. By using clonally related isolates and genetically defined mutants, we determined the fitness of FQ-resistant \textit{Campylobacter} in chicken (a natural host and a major reservoir for \textit{C. jejuni}) in the absence of antibiotic selection pressure. When mono inoculated into the host, FQ-resistant and FQ-susceptible \textit{Campylobacter} displayed similar levels of colonization and persistence in the absence of FQ antimicrobials. The prolonged colonization in chickens did not result in loss of the FQ resistance and the resistance-conferring point mutation (C257 \rightarrow T) in the \textit{gyrA} gene. Strikingly, when co inoculated into chickens, the FQ-resistant \textit{Campylobacter} isolates outcompeted the majority of the FQ-susceptible strains, indicating that the resistant \textit{Campylobacter} was biologically fit in the chicken host. The fitness advantage was not due to compensatory mutations in the genes targeted by FQ and was linked directly to the single point mutation in \textit{gyrA}, which confers on \textit{Campylobacter} a high-level resistance to FQ antimicrobials. In certain genetic backgrounds, the same point mutation entailed a biological cost on \textit{Campylobacter}, as evidenced by its inability to compete with the FQ-susceptible \textit{Campylobacter}. These findings provide a previously undescribed demonstration of the profound effect of a resistance-conferring point mutation in \textit{gyrA} on the fitness of a major foodborne pathogen in its natural host and suggest that the rapid emergence of FQ-resistant \textit{Campylobacter} on a worldwide scale may be attributable partly to the enhanced fitness of the FQ-resistant isolates.

Luo et al., 2005, PNAS
Role of Calf-Adapted Escherichia coli in Maintenance of Antimicrobial Drug Resistance in Dairy Calves

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Antimicrobial Drug Resistance Genes Do Not Convey a Secondary Fitness Advantage to Calf-Adapted Escherichia coli

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Bacterial Fitness and Resistance

FIG. 4. In vivo competition experiments in neonatal calves \((n = 6)\) between \(\text{Na}^{+} \text{SSuT}\) and \(\text{Na}^{+}\) susceptible strains. Each circle represents a sample from a single calf. \(\text{CI} = (X - Y)/(X + Y)\) where \(X\) is the number of SSuT colonies and \(Y\) is the number of susceptible colonies. When \(\text{CI} = 0\), there is an equal proportion of \(\text{Na}^{+} \text{SSuT}\) and \(\text{Na}^{+}\) susceptible strains in the sample.

FIG. 5. In vivo competition experiments in heifers \((n = 12)\) between \(\text{Na}^{+} \text{SSuT}\) and \(\text{Na}^{+}\) susceptible strains. Each circle represents a sample from a single heifer. \(\text{CI} = (X - Y)/(X + Y)\) where \(X\) is the number of SSuT colonies and \(Y\) is the number of susceptible colonies. When \(\text{CI} = 0\) there is an equal proportion of \(\text{Na}^{+} \text{SSuT}\) and \(\text{Na}^{+}\) susceptible strains in the sample.
FIG. 2. Competition experiments in neonatal calves: for the control group ($n = 3$), competition between SSuT NaI' and wild-type susceptible NaI strains (open circles), and for the experimental group ($n = 6$), competition between SSuT NaI' and cured-SSuT (susceptible) NaI' strains (closed circles). Each circle represents the mean with the 95% confidence interval bars for the time point shown on the x axis. CI = $(X - Y)/(X + Y)$, where $X$ is the number of resistant colonies and $Y$ is the number of susceptible colonies. When CI is 0, there is an equal proportion of the competing resistance patterns.
Co-resistance

- When one organism is resistant to multiple classes of antibiotic (MDR)
- Can be due to one genetic mechanism (efflux pump)

Co-selection

- When one compound can select for resistance to another compound
Multidrug Resistance Plasmids

Call et al., Antimicrob. Agents Chemother., 2010
Multidrug Resistance Plasmids

Fernandez-Alarcon et al., PLoS ONE, 2011
Short communication

Decreased susceptibility to zinc chloride is associated with methicillin resistant *Staphylococcus aureus* CC398 in Danish swine

Frank Møller Aarestrup *, Lina Cavaco, Henrik Hasman

*National Food Institute, Technical University of Denmark, Bülowsvej 27, DK-1790 Copenhagen V, Denmark*
Long-term follow-up study

Use of resistance gene copy number as a better reflection of selection pressure over time

Four dairies with up to 72 animals per farm followed for 2.5 years (sampled every 3 mos.)

Hypothesis: Antibiotic resistance gene levels would increase over time, faster in herds with higher antibiotic use
<table>
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<th>mefA</th>
<th>ermB</th>
<th>tetM</th>
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<tr>
<td>Time</td>
<td>0.08</td>
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## Low Copy Genes

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<td><strong>Time</strong></td>
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Log_{10} Gene Copy over Time

- **Gene Copy per Gram**
  - mefA
  - tetM
  - ermB
  - tetA
  - flo
  - cmy

**Log_{10} Copies over Time**

Time:

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
Gene levels on farms did not track with farm-level use of antibiotics

Age of animal (very young animal) continues to be associated with increased antibiotic resistance detection

qPCR may be a more sensitive method for detecting the effect of selection pressures on resistance than cultivation
Take Home Messages

- We need a unified definition of resistance
  - Resistance has a clinical interpretation
  - Decreased susceptibility for non-clinical cut-offs?
  - Harmonization of cut-offs

- Resistance is not new
  - Anthropogenic factors may enhance spread and assembly of multidrug resistance
**Take Home Messages**

- Relationship between antibiotic use and resistance may require:
  - Long-term pressure (decades?)
  - High density of selection (whole herd versus individuals)
  - Specific doses

- Be prepared for the unintended consequences
  - Hard to predict the diversity of pressures that can select for resistance
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