Antimicrobial Resistance

Molecular Genetics of Antimicrobial Resistance

- Micro evolutionary change - point mutations
  - Beta-lactamase mutation extends spectrum of the enzyme
  - *rpoB* gene (RNA polymerase) mutation alters rifampin binding site preventing activity
- Macro evolutionary change - rearrangements of segments of DNA
  - Transposons carrying antibiotic resistance genes
- Acquisition of foreign DNA - plasmids, phage *etc.*
  - Conjugative plasmids can transfer resistance genes between different species

Consequences of Antimicrobial Resistant Bacteria

- Change in the approach to the administration of "empiric antimicrobial therapy"
- Increased number of hospitalizations
- Increased length of hospitalization
- Increased morbidity and mortality
  - Emergence of strains totally resistant to all available antimicrobials
- Choice of more expensive or more toxic therapeutic alternatives

Molecular Genetics of Antimicrobial Resistance

- Cross resistance - often (but not always) a single resistance mechanism confers resistance to an entire class of antibiotics
  - Methicillin resistance confers resistance to all beta-lactams penicillins and cephalosporins
- Cross resistance among different classes of antibiotics can occur as the result of:
  - Drug efflux pumps
  - Overlapping targets *e.g.* macrolides and lincosamides share overlapping targets on the ribosome that can be altered by methylation of an adenine residue

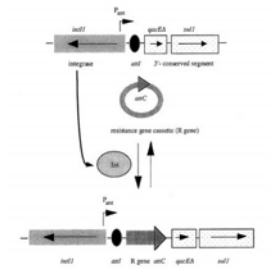
Topics to be Covered

- Molecular genetics of antimicrobial resistance
- Mechanisms of antimicrobial resistance
- Dissemination of antimicrobial resistance genes
- Epidemiology of antimicrobial resistance
  - Antibiotic use in the community
  - Antibiotic use in agriculture
  - Antibiotic use in hospitals
- Measures to reduce the spread of antimicrobial resistance

Molecular Genetics of Antimicrobial Resistance

- Co-resistance refers to the presence of several resistance mechanisms in the same organism
- Co-selection refers to the selection of multiple antibiotic resistance genes when one resistance mechanism is selected. Generally occurs if both genes are regulated by the same promoter
- Integrons are mobile genetic elements present in both Gram positives and negatives that mediate both co-resistance and co-selection

Integrons

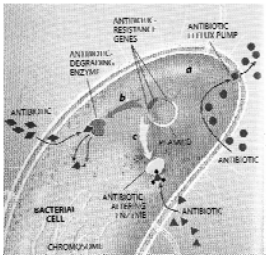


- Coordinately express genes under the control of a single promoter
- Represent "hot spots" for site-specific recombination allowing integration of nonhomologous sequences
- Gene transcription is correlated with the proximity of the gene to the promoter

Antibiotic Degrading Enzymes β-lactamases

- Gram positive beta-lactamases
  - Primarily found in staphylococci
  - Excreted extracellularly
  - Usually plasmid-mediated, often packaged with other antimicrobial resistant determinants *e.g.*, aminoglycosides
- Gram negative beta-lactamases
  - Large variety of different beta-lactamases carried by many Gram negative species with different spectrum of activity
    - Extended spectrum beta-lactamases - plasmid mediated, broad spectrum *Klebsiella spp.* among the first to carry these ESBLs
  - Secreted into the periplasmic space
  - Can be chromosomal or plasmid
  - Single point mutation can change the substrate specificity

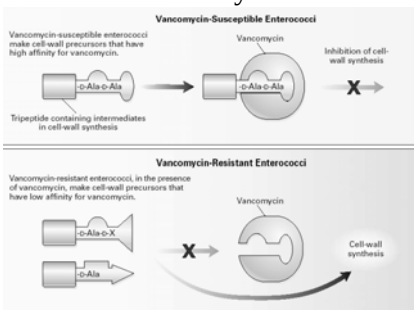
Mechanisms of Antimicrobial Resistance



Levy, Scientific American

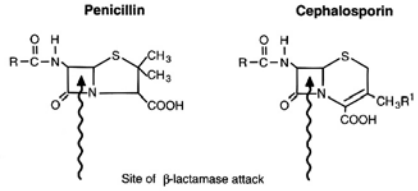
- Enzymatic modification
  - Beta lactamases
- Decreased accumulation of antibiotic
  - Permeability barriers - outer membrane Gram negatives - PCNs
  - Porin mutations - carbapenems
  - Antibiotic efflux pumps - tetracyclines, macrolides
- Alteration of the drug target
  - Methicillin, vancomycin, macrolides

Alteration of the Drug Target Site Vancomycin



Murray, NEJM

Enzyme Modifiers - Beta-lactamases

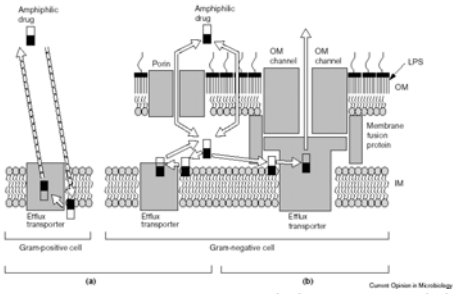


Splits the amide bond hydrolyzing the β-lactam ring

Alteration of the Drug Target Site

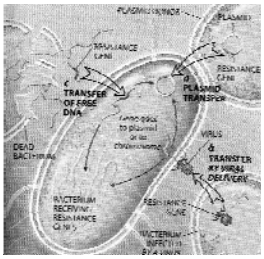
- Enterococcal resistance to vancomycin - different types but all involve synthesis of altered cell wall precursor side chain that doesn't bind vancomycin *e.g.*, D-Ala-D-Lactate vs. D-Ala-D-Ala
  - This mechanism may be plasmid-mediated
- Staphylococcal resistance to semisynthetic penicillins
  - Synthesis of a novel penicillin binding protein (2a) with reduced affinity for methicillin
  - Takes over role of other PBPs (which are methicillin susceptible) in cell wall synthesis

Antibiotic Efflux Pumps



Nikaido, Curr. Opin. Microbiol.

Acquisition of Foreign DNA



Levy, Scientific American

- Horizontal gene transfer is common, even between Gram positive and negative bacteria
- Plasmid - transfer of single or multiple resistance genes
- Free DNA - acquisition of resistance genes by naturally transformable species *e.g.*, pneumococcus, neisseria
- Bacteriophage - virus mediated transfer of genes

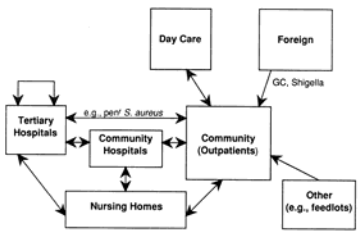
Antibiotic Efflux Pumps

- Originally designed to protect organism from toxic material therefore have broad substrate specificity
- There are a variety of different efflux systems used by bacteria although the majority use proton-motive force as the means for efflux
- They can pump out a wide variety of different molecules including tetracyclines, beta-lactams, detergents, macrolides and quinolones
- Transfer of this type of resistance is not easy because of the complex genetic machinery needed for the pump to be functional

Community Acquired Resistant Pathogens: Penicillin-Resistant Pneumococcus

- Historical perspective - 1st large scale outbreak in Durban South Africa - 1977
  - Associated with children < 5 years, measles complicated by pneumonia and antimicrobial therapy
  - Isolates also resistant to tetracycline, chloramphenicol
- Nature of the resistance
  - Decreased affinity for penicillin-binding proteins
    - PBP 2b
  - Structurally altered cell wall

Epidemiology of the Transmission of Antibiotic-Resistant Bacteria



Community Acquired Resistant Pathogens: Penicillin-Resistant Pneumococcus

- Acquisition of resistance
  - Uptake of foreign DNA (*e.g. S. mitis*) by naturally competent *S. pneumoniae*
  - Recombination event leading to replacement of susceptible PBP with resistant one
- Clonal dissemination
  - Expansion of a limited number of clones (perhaps as a result of other associated virulence determinants)
  - Association of carriage with young children, crowded settings
  - International spread - vacations in hot spots, selective antibiotic pressure

International Spread of Resistant Clones of Pneumococcus

Dowson, Trends Microbiol

Antibiotics in Agriculture

- Antimicrobials are routinely added to animal feed and water to promote animal growth
  - Rationale is to promote more rapid growth reducing farming expenses
  - Mechanisms are debated although most commonly invoked is the reduction of infections, especially in unsanitary conditions
- Many of the antibiotics used in this setting are of the same class as those used to treat human infections
  - Macrolides, tetracyclines, glycopeptides

Antibiotics in Agriculture

The Example of Vancomycin Resistance

- In Europe *E. faecium* resistance to vancomycin (VRE) described in 1986
- The use of the glycocpeptide, avoparcin, in animal feed is believed to be responsible for the emergence of VRE
- Animal reservoir as a source of VRE is supported by epidemiologic data
  - In Denmark 24 kg of vanco used for humans vs. 24,000 kg for animals
  - VRE found in avoparcin fed animals (dead or alive!)
  - VRE types in animals and humans related by molecular typing

Antibiotics in Agriculture

Subject	**Antimicrobial (Pounds)**
Human	3,000,000
Beef*	3,700,000
Swine*	10,300,00
Chicken*	10,500,000
Total in animals	**24,500,000**

* Nontherapeutic uses only, 1990's

Union of Concerned Scientists

Antibiotics in Agriculture: Effect on Animals and Farmers

CHANGES IN INTESTINAL FLORA OF FARM PERSONNEL AFTER INTRODUCTION OF A TETRACYCLINE-SUPPLEMENTED FEED ON A FARM

STUART B. LEVY, M.D., GEORGE B. FITZGERALD, PH.D., AND ANN B. MACONE, B.S.

Abstract A prospective study was undertaken to determine whether feeding farm animals antibiotics in feed caused changes in the intestinal bacterial flora of farm dwellers and their neighbors. Chickens were fed tetracycline-supplemented feed (tet-feed), and, as expected, within one week their intestinal flora contained almost entirely tetracycline-resistant organisms. Increased numbers of resistant intestinal bacteria also appeared, but more slowly, in farm members, but not their neighbors. Within five and six months, 31.3 per cent of weekly fecal samples from farm dwellers contained >80 per cent tetracycline-resistant bacteria as compared to 6.8 per cent of the samples from the neighbors (P<0.001). Seven of the 11 farm members, but only three of the 24 neighbors, had two or more fecal samples containing >80 per cent tetracycline-resistant coliforms (P<0.01). These resistant bacteria contained transferable plasmids conferring multiple antibiotic resistances. Selective pressure by tet-feed for antibiotic-resistant bacteria in chickens extends to human beings in contact with chickens and the feed. (N Engl J Med 295:583-588, 1976)

Antibiotics in Agriculture: Effect on Animals and Farmers

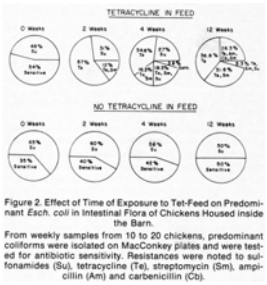
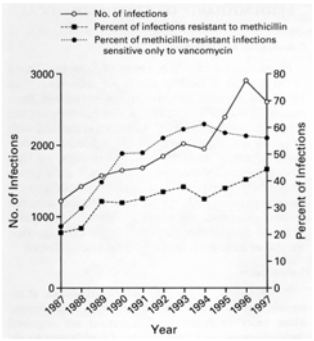


Figure 2. Effect of Time of Exposure to Tet-Feed on Predominant *Esch. coli* in Intestinal Flora of Chickens Housed inside the Barn.

From weekly samples from 10 to 20 chickens, predominant coliforms were isolated on MacConkey plates and were tested for antibiotic sensitivity. Resistances were noted to sulfonamides (Su), tetracycline (Te), streptomycin (Sm), ampicillin (Am) and carbenicillin (Cb).

- Multiple resistance found in >50% *E. coli* in chickens receiving tetracycline >10 wks
- A similar observation was made in farm dwellers but not in neighbors over time

Antimicrobial Resistance - *S. aureus*



Antibiotics in Agriculture: Transmission to Humans

AN OUTBREAK OF MULTIDRUG-RESISTANT, QUINOLONE-RESISTANT *SALMONELLA ENTERICA* SEROTYPE TYPHIMURIUM DT104

KARE MOLBAK, M.D., DORTE LAU BAGGESEN, D.V.M., PH.D., FRANK MØLLER AARESTRUP, D.V.M., PH.D., JENS MUNK EBBESEN, D.V.M., JØRGEN ENGBERG, M.D., KAI FRYDENDAHL, D.V.M., PETER GERNER-SMIDT, M.D., D.MED.SCI., ANDREAS MUNK PETERSEN, M.D., AND HENRIK C. WEGENER, PH.D.

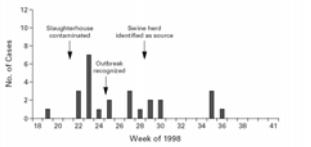
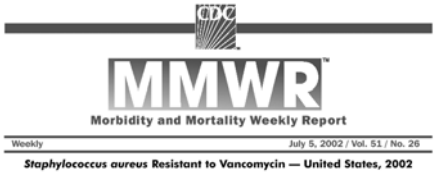


Figure 1. Number of Cases of Quinolone-Resistant, Multidrug-Resistant *Salmonella enterica* Serotype Typhimurium DT104 in Denmark, According to Week of Onset, May through August 1996. The numbers on the x axis indicate the weeks of the year.

First Clinical VRSA Reported in USA



***Staphylococcus aureus* Resistant to Vancomycin — United States, 2002**

Nosocomial Infections - *Staphylococcus aureus*

- Penicillin first introduced in the early 1940's followed shortly thereafter by the detection of penicillin resistance
  - Due to beta-lactamase
- Semisynthetic penicillins introduced in the early 1950's followed by the emergence of methicillin-resistance
  - Due to altered penicillin binding protein
  - Epidemic spread of MRSA clones world wide
  - Vancomycin the sole bactericidal agent to treat these infections
- Emergence of MRSA with reduced susceptibility to vancomycin
  - Altered cell wall "sponge hypothesis"

Control of the Spread of Antimicrobial Resistance

- Eliminate the use of antimicrobials in animal feed
- Restrict use of antibiotics for inappropriate indications
  - Upper respiratory infections
- Antibiotic restriction in hospital settings
  - Antibiotic cycling?
- Enforce infection control policies
  - Handwashing *etc.*
  - Appropriate isolation procedures

So What Should You Know

- Molecular genetics of resistance - terminology
  - Integrons
- Mechanisms of antimicrobial resistance
- Mechanisms of dissemination of antimicrobial resistant strains
- Examples of resistance phenomena in the community, hospital setting
  - Including agricultural settings

Evolution of Antimicrobial Therapy in a Nutshell

Year 2000 B.C. "Here, eat this root."
Year A.D. 1000 "That root is heathen.
Here, say this prayer."
Year 1850 "That prayer is superstitious.
Here, drink this potion."
Year 1920 "That potion is snake oil.
Here, swallow this pill."
Year 1945 "That pill is ineffective.
Here, take this penicillin."
Year 1955 "OOPS, bugs mutated.
Here, take this tetracycline."
Years 1960–1999 Thirty-nine more OOPS's.
"Here, take this more powerful antibiotic."
Year 2000 The bugs have won! "Here, eat this root."

Anonymous observation from the *World Health Report on Infectious Diseases, 2000*