Antimicrobial Chemotherapy & Resistance

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October, 2008

Learning Objectives

• To understand how antibiotics work
• To review how drug resistance develops in bacteria
• To summarize current status of antimicrobial resistance in selected bacteria
• To understand how resistant organisms are detected in the clinical laboratory
Terminology

- **Antibiotic** - An agent that can be naturally occurring, partially or completely synthetic that selectively inhibits the growth of microorganisms at low concentrations (penicillin)
- **Antiseptic** – An agent used to inhibit or eliminate microbes on skin or other living tissue (alcohol, iodine, chlorhexidine)
- **Disinfectant** – An agent used to destroy microbes on inanimate objects (phenols, formaldehyde, chlorine bleach)

Sir Alexander Fleming

Discovered Penicillin in 1928
Agents Acting on Cell Wall: Beta Lactams

- Penicillins
- Penicillinase-stable
- Broad-spectrum
- Beta-lactamase Inhibitor combinations

Beta lactam ring resembles substrate for penicillin binding proteins (enzymes)

Penicillin Mechanism

- Binds active site of transpeptidase enzyme that cross-links peptidoglycan by joining D-ALA and GLY
- Mimics D-alanyl-D-ALA that would normally bind to this site
- Irreversibly inhibits transpeptidase
- Growth of the bacterial cell wall is inhibited
Other Beta Lactams

- Cephalosporins 1-4\textsuperscript{th} generations
- Monobactams
- Carbapenems

Non-Beta Lactams Acting on Cell Wall

- Cycloserine
  - Inhibits synthesis of D-alanyl-D-ALA within cell call mucopeptide
- Glycopeptides (vancomycin)
  - binds to D-ALA-D-ALA moiety of precursor subunit blocking transpeptidation
- Bacitracin
  - complexes with lipid carrier that transports peptidoglycan precursors from cytoplasm to cell membrane
- Isoniazid (INH)
  - Inhibits fatty acid and lipid components of mycolic acid synthesis in mycobacteria
Examples of Drugs Acting on Bacterial Ribosome

- **Tetracyclines** prevent attachment of tRNA-AA to 30s.
- **Aminoglycosides** bind 30s, inactivate initiation complex, misread mRNA genetic code & prematurely release peptide.
- **Macrolides** bind 50s & prevent release of deacylated tRNA, preventing peptide elongation.
- **Chloramphenicol & clindamycin** bind 50s & prevent peptide bond formation.
- **Oxazolidinones** bind 50s & prevent formation of initiation complex.
- **Streptogramins** bind 50s, prevent peptide elongation and premature release from ribosome.
- **Tetracyclines** prevent attachment of tRNA-AA to 30s.

Agents Interfering with Cell Membranes

- Polymyxins
- Daptomycin
Agents Interfering with DNA Replication

- **Rifampin** – binds DNA-dependent RNA polymerase & prevents mRNA transcription
- **Metronidazole** – intermediate metabolites damage DNA in anaerobes
Antimetabolites

- Sulfonamide
  - inhibits dihydropteroate synthase
  - disrupts folic acid synthesis
- Trimethoprim
  - inhibits dihydrofolate reductase
  - synergy with sulfonamide
“Chemotherapy without bacteriology is guesswork”

Terminology

- **MIC** – minimum inhibitor concentration
  - the lowest concentration of antimicrobial that inhibits visible bacterial growth
- **MBC** – minimum bactericidal concentration
  - the lowest concentration of antimicrobial that kills 99.9% of an inoculum
Susceptibility Testing Methods:
Agar Disk Diffusion

- Rapidly growing bacteria
- Qualitative
- Inverse relationship between zone size & MIC
- CLSI sets criteria used by labs to interpret

Agar Gradient Diffusion (Etest)

- Quantitative (MIC)
- Use for many types of organisms
- MIC read where ellipse of inhibition intersects plastic strip containing gradient of drug
Automated Bacterial Identification and Susceptibility Testing Systems: MicroScan WalkAway 96

Microbroth Dilution Susceptibility Testing
Agar Dilution

- Reference method
- Too time consuming and expensive for routine use as primary method
- Excellent for screening single drugs
  - MRSA (oxacillin screen agar)
  - Vancomycin-resistant enterococcus agar

Lab Report Terminology

- **Susceptible** - appropriate Rx with recommended dosage
- **Intermediate** - MIC approaches blood/tissue level for which response may be less than for susceptible isolates. May Rx for infection in sites where drug is concentrated or if high dose is used
- **Resistant** - organism not inhibited by achievable systemic concentration with normal dosage and/or ralls in range where clinical efficacy unreliable
Desirable Properties of Antibiotics

- Selective toxicity
- Water soluble
- Bactericidal
- High serum levels achieved for several hours
- Broad spectrum
- Minimal effect on normal flora
- Low potential for inducing resistance
- Minimal side effects/toxicity

Clinical Pharmacology of Antibiotics

- Serum/tissue concentrations
- Route of elimination
- Half life
- Oral vs. parenteral
- Duration of treatment
- Bacteriostatic vs. bactericidal
- Kinetics of killing
  - Concentration vs. time dependent
Indications for Cidal Drugs

- Life threatening conditions
  - Endocarditis
  - Meningitis
  - Immunosuppressed host

Combination Therapy

- Broad spectrum
- Polymicrobial infections
- Prevent resistance
- Synergy

- Risk of toxicity
- Superinfection
- Cost
- Antagonism
Antimicrobial Prophylaxis

• Prevent endocarditis following dental work
• Contacts of meningococcal meningitis
• Opportunistic infections in AIDS
• Recurrent UTIs
• Prevent post-surgical infections
“Antibiotic resistance has been a fact of life since the dawn of the antibiotic era in the mid 20th century. Clinical deployment of every new antimicrobial agent has been greeted with the development of resistance in one or more bacterial species.”

“Environmental conditions enhance ability of bacteria to develop resistance and proliferate. Ability to survive may be the result of spontaneous mutation or acquisition of new DNA. Organisms with new mutations or genes probably would not survive if it were not for environmental conditions that encouraged their emergence.”

FC Tenover
Hosp Pract, Feb, 1999
Potential Routes for Spread of Drug-Resistant Bacteria

Other Factors That May Increase Antimicrobial Resistance in Hospitals

- Greater severity of illness
- Immunocompromised patients
- New devices and procedures
- Ineffective infection control practices
- Increased use of antimicrobial prophylaxis
- Empiric polymicrobial antimicrobial therapy
Organism Characteristics Favoring Resistance

- Intrinsic resistance to some drugs
- Ability to exchange genetic information
- Ability to survive adverse environmental conditions
- Easily colonize, infect, and transmit
- Reservoirs in body

Impact of Antimicrobial Resistance

- Prolonged illness/hospitalization
- Increased mortality
- Inappropriate therapy
- More expensive/toxic therapy
- More lab tests
- Spread of infectious organisms that may have no effective treatment and that may be impossible to eradicate from hospital
How Does Antibiotic Resistance Affect the Biology of the Bacteria?

- It does not typically make the organism more virulent in its ability to cause disease or produce pathologic lesions in the host.
- Slight resistance may still be overcome with higher doses of antibiotic, especially if the drug is concentrated in the site of infection.
- Highly resistant strains will not respond and will require alternative treatments.

Types of Antimicrobial Resistance

- Innate or Primary
- Acquired
How Bacteria Pick Up Resistance Genes

Several well-characterized PRSP clones have spread worldwide, driven by selective antibiotic pressure.
Up to 85% of PRSP in U.S. belong to one of 9-10 clonal groups, often MDR.

Clonal spread of *S. pneumoniae* Spain^{23F}

Bacterial Strain Typing PFGE

PFGE patterns and dendrogram of a single clone of vancomycin-resistant *E. faecium* isolated from 14 patients at UAB. 5 isolates were from 1997; 8 isolates were from 1998; and 1 isolate was from 1999 indicating persistence of infecting organisms in the nosocomial environment for many months.

Mechanisms of Antimicrobial Resistance

- **Decreased permeability**
  - Aminoglycoside
- **Active Efflux**
  - Erythromycin
- **Altered Target**
  - Fluoroquinolones
- **Bacterial Defense Mechanism**
  - Bypass
  - Trimeth/sulfa
- **Enzyme Inactivation**
  - Penicillin

*Ref: Murray 1991.*
**β-Lactams: Mechanisms of Resistance**

- **β-Lactamases**
- **Susceptible**
- **Resistant**

**Penicillin-binding proteins**

**Mechanisms of Resistance to Fluoroquinolones**

- Efflux pump is a less potent and less common cause of resistance
- Mutation of bacterial genes for binding sites causes resistance
Macrolides: Mechanisms of Resistance

- Drug efflux
- Esterases
- Ribosome
- Susceptible
- Resistant

Sulfonamide Resistance: Metabolic Bypass

- Resistance occurs when bacterial strains develop alternative enzyme systems to synthesize folic acid
- Sulfonamides inhibit folic acid synthesis by binding to dihydropteroate synthase, a critical step in DNA synthesis
Methicillin resistant *Staphylococcus aureus* (MRSA)

Problematic resistance in nosocomial infections:

- *Staphylococcus aureus*
  - skin and wound infections
  - bacteremia
- > 50% of *S. aureus* are MRSA in many hospitals
- Carrier spread within and between institutions by healthcare workers
- Most MRSA are resistant to almost all drug classes except vancomycin
- Community-acquired MRSA becoming more common

**MRSA: An Escalating Problem**

UAB Hospital (1996-2006)

MRSA attack rate: 5/1000 admissions
ESBLs: Plasmid-Mediated Resistance

Transposons: (mobile genetic elements)

Transferable Plasmid carries genetic information, including codes for various resistance factors

Controlling Drug Resistance

- Education
- Healthcare providers and consumers
- Limit unnecessary antibiotics
- Proper dose and duration of therapy
- Limit prophylaxis
- Target pathogens
- CDC guidelines