Mechanisms of Antibiotic Resistance

I. PROBLEM OF RESISTANCE

A. Resistance varies with setting, e.g. hospital vs. community

B. Resistance varies with geographical location

C. Multiple antibiotic resistance - *Staph. aureus*, *Strep. pneumoniae,* tuberculosis

D. Infectious resistance - infection may be in abcess or at intracellular location

II. MOLECULAR GENETICS OF ANTIBIOTIC RESISTANCE

Bacteria are rapidly growing organisms. A typical infection that causes symptoms will contain 10^9 to 10^{12} bacteria. Based on normal genetic variability, this population of bacteria will have a wide variability of response to an individual antibiotic. In the face of antibiotic pressure, genetic mutations leading to resistance are a necessary and likely outcome for the bacteria i.e. "survival of the fittest".

A. Chromosome-mediated resistance - Spontaneous mutations

1. Frequency of spontaneous mutations is 10^7 -10^9. Low frequency is unrelated to presence of antibiotic.

2. Not a major reason for massive sudden emergence of drug resistance

3. Mutations rarely lead to complete resistance

4. If mutation is stable, selection pressure will rapidly increase the numbers of drug resistant mutants.

B. Plasmid-Mediated Resistance - "Conjugation"

1. Importance from a clinical standpoint

a. Occurs in many different species, especially Gram – rods.
50% of GI tract inhabitants can transfer resistant plasmids (R-factors).

b. Plasmids frequently mediate resistance to multiple drugs

c. Plasmids have a high rate of transfer from one cell to another.

2. Mechanism of plasmid-mediated resistance

a. Definitions

Plasmid – non-essential, extrachromosomal, self-replicating element composed of circular, double-stranded DNA.

Episome - DNA that can integrate into chromosomal DNA

R-factor – a plasmid that encodes for antimicrobial resistance

b. R-factors (resistance factors)

i. Transfer occurs during mating to drug sensitive recipient bacteria

ii. Replicate independently. Cell may contain multiple copies.

iii. Can be transferred to bacteria of other species and genera

iv. Two sizes. Large plasmids (MW = 10 million) are conjugative R-factors that contain extra DNA for conjugation process. Small R-factors only contain resistance genes.

3. Transposon-mediated resistance - "Transduction" & "Transformation"

a. Definition

Transposon - resistance genes that are transferred within or between large pieces of either chromosomal DNA or plasmids.

b. Transduction

i. phage-mediated transfer of resistance genes. A phage is a virus that infects bacteria. During lysogeny (bursting of cell releasing many copies of phage), phage which pick up resistance genes that can infect antimicrobial sensitive cells.

ii. Clinically important, especially for Gram + bacteria e.g. *Staph.*

c. Transformation

i. Uptake of resistance transposon by a sensitive bacterium after lysis of a resistant bacteria.

ii. not of major clinical significance

III. SPECIFIC MECHANISMS OF RESISTANCE

A. Inactivation of drug by enzymes - usually plasmid-mediated

1. Penicillins & Cephalosporins - β-lactamases (penicillinases)
2. Aminoglycosides - phosphorylation, adenylation, or acetylation
3. Chloramphenicol – acetylation
4. Macrolides - erythromycin esterase

B. Alteration of membrane permeability

1. Common mechanism in Gm – bacteria. Change in porins or transport proteins.
2. Examples - tetracyclines, β-lactams, aminoglycosides, quinolones

C. Efflux pumps – Active transport pump to remove antimicrobial agent

1. Active transport or efflux pumps tetracyclines, quinolones, and macrolides out of bacteria

D. Alteration of intracellular target site

1. Macrolides - methylation of 23S ribosomal RNA, blocking erythromycin binding
2. Aminoglycosides - altered protein in 30S ribosome

E. Alteration of intracellular target enzyme

1. Trimethoprim - production of dihyrofolate reductase with high Km
2. β-lactams - alteration in pencillin binding proteins with less affinity or decreased production of PBPs.
3. Rifampin - altered DNA dependent RNA polymerase
4. Quinolones - modified DNA gyrase and topoisomerase IV

F. Overproduction of target enzyme

1. Sulfonamides - increased levels of dihydropteroate synthetase
2. Trimethoprim - increased levels of DHFR

G. Auxotrophs that bypass blocked step

1. Sulfonamides – Resistant auxotrophs can utilize exogenous folic acid.
2. Trimethoprim - loss of thymidylate synthetase. Reistant bacteria take up thymidine and produce thymidylate via salvage pathways.

H. Absence of autolytic enzymes

1. β-lactams activate murein hydrolase (autolysin), an enzyme that breaks down the peptidoglycan. Tolerant cells lack this enzyme.

IV. CONTROL OF RESISTANCE

A. Give the optimal antibiotic

1. Is it necessary ?

2. Is the pathogen sensitive ?

3. Will the drug get to the site of infection ?

4. Are therapeutic concentrations achieved at the site of infection ?

5. Is toxicity acceptable (risk vs. benefit)

6. Is the therapy cost effective ?

B. Administer high antibiotic loading doses for antibiotics that display concentration-dependent killing. e.g a one-time dose for STDs or single dose daily therapy for aminoglycosides.

C. Stress good patient compliance - directly observed therapy for STDs & tuberculosis

D. Simultaneous therapy with unrelated antibiotics - Synergy & decreased chance of resistance.

E. Use antibiotics only when necessary -strict formulary review & use criteria

F. Place absolute limits on use of certain antibiotics e.g. quinolones ?, amikacin, linezolid

G. Reduce antibiotic exposure - animal feeds, self-limiting infections, third-world use

H. Randomly rotate use of different antibiotics - limits continual exposure in institution

I. Use inhibitors of inactivating enzymes ?

Table IV. Mechanisms of Resistance

The Pathogens - "Know Thine Enemy"

Table 5. Major Bacterial Pathogens

Type of Organism	Genus	Disease
Readily Gram stained		
Gram positive cocci	*Staphylococcus aureus*	Skin and tissue abcesses, osteomyelitis, pneumonia enterocolitis, toxic shock
	Staph. epidermidis	endocarditis, prosthetic inf.
	Streptococcus pyogenes	cellulitis, pharyngitis
	Strep. pneumoniae	Pneumonia, meningitis, otitis media, sinusitis
	Enterococcus	endocarditis, UTIs
Gram negative coccobacilli	*Haemophilus influenzae*	otitis media, sinusitis, bronchitis, meningitis
	Neisseria,	gonorrhea, meningitis
	Moraxella catarrhalis,	otitis media, bronchitis, sinusitis
Gram positive rods	*Corynebacterium*	diphtheria
	Listeria,	meningitis
	Bacillus	anthrax, food poisoning
	Clostridium	tetanus, gas gangrene, botulism
	Actinomyces	actinomycosis, pneumonia
	Nocardia	pneumonia, meningitis
Gram negative rods - facultative aerobes		
Enteric tract organisms		
Pathogenic inside & outside GI tract	*Escherichia*	UTIs, diarrhea, pneumonia
	Salmonella	enterocolitis, typhoid fever
Pathogenic primarily inside GI tract	*Shigella*	enterocolitis
	Vibrio	cholera
	Campylobacter	enterocolitis
	Helicobacter	gastric ulcers
	Aeromonas	enterocolitis, wound infections
Pathogenic outside GI tract	*Klebsiella-Enterobacter-Citrobacter-Serratia* group,	pneumonia, UTIs
	Proteus-Providencia-Morganella group,	UTIs
	Pseudomonas-Acinetobacter-Xanthomonas group	pneumonia, UTIs
Gram negative rods - anaerobes	*Bacteroides*	peritonitis
Respiratory tract organisms	*Haemophilus*	otitis media, pneumonia
	Legionella,	pneumonia
	Bordetella	pertussis
Organisms from animal sources	*Brucella*	brucellosis
	Franciscella	tularemia
	Pasteurella	cellulitis
	Yersinia	bubonic plague

UTIs = urinary tract infections

Type of Organism	Genus	Disease
Not Readily Gram Stained		
Non-obligate intracellular parasites	*Mycobacterium* *Mycoplasma* *Treponema* *Leptospira* *Borrelia*	tuberculosis, leprosy pneumonia syphilis leptospirosis Lyme disease
Obligate intracellular parasites	*Chlamydia* *Rickettsia*	urethritis, PID, pneumonia Rocky Mt. spotted fever, typhus, Q fever

PID = pelvic inflammatory disease

Table 6. *Top Notifiable Bacterial Diseases in U.S. 2000*

Infectious Disease	Number of cases per year
Chlamydia	642,588
Gonorrhea	335,098
Lyme disease	13,309
Tuberculosis	12,942
Pertussis	6,755
Syphilis, primary & secondary	5,894
E. coli O157:H7	4,410
Cryptosporidiosis	2,573
Meningococcal infections	2,035
Malaria	1,288
Legionellosis	1,249
Haemophilus infections (invasive disease)	982
Listeriosis	662

Note: The numbers provided above are those reported to State Departments of Health or the CDC. Data was collected through the 52th week (Dec. 30th 2000). It is likely that these numbers are significantly under-reported. More common bacterial diseases such as streptococcal pharyngitis are not required to be reported to the CDC. In 2000, NETSS reported 36,762 cases of Salmonellosis and 20,721 cases of Shigellosis. Nationwide reporting for *Chlamydia* began in 1996.

For *Strep. pneumoniae,* the number of infections per year in the U.S. has been estimated as follows:

Meningitis	3,000
Bacteremia	50,000
Pneumonia	500,000
Otitis media	7,000,000

Reference: J. Inf. Dis. 116:1346-53 (1992).

Chlamydia trachomatis is the most prevalent sexually transmitted disease with 4 million new cases per year in the U.S.

Reference: MMWR 42:1-39 (1993).

Table 7. Bacteria Associated with Human Disease

Organ System	*Common pathogens*	*Uncommon pathogens*
Blood (bacteremia)	Coagulase negative *Staph., Staph. aureus, Strep. pneumoniae*, other *Strep. sp., Enterococcus, H. influenzae, N. meningitidis, E. coli, Klebsiella pneumoniae, Enterobacter, Proteus mirabilis*, other Enterobacteriaciae, *Ps. aeruginosa*, other *Ps. sp., Burkholderia, Bacteroides fragilis*	Many aerobic and anaerobic bacteria
Heart (endocarditis) Native valve	*Viridans* group streptococci, *Enterococcus, Staph. aureus, Pseudomonas*	*Strep. pneumoniae*, HACEK group
Prosthetic valve	Coagulase negative *Staph., Staph. aureus, Enterococcus, Corynebacterium sp.*	*Strep. pneumoniae, Mycobacterium chelonae*
Central Nervous System		
Acute meningitis (infants)	*Strep. pneumoniae, N. meningitidis, and Haemophilus influenzae* (rapidly decreasing)	*Leptospira, Staph. aureus*
Neonatal meningitis	group B *Strep., E. coli, Listeria monocytogenes*	
Chronic meningitis	*Mycobacterium tuberculosis, Nocardia, Treponema pallidum*	*Borrelia burgdorferi, Brucella,* other *Mycobacterium sp.*
Brain abcess	*Viridans* group streptococci, mixed anaerobes *(Bacteroides, Fusobacterium, Porphyromonas, Prevotella, Peptostreptococcus), Staph. aureus*	*Clostridium sp., Haemophilus, Nocardia,* Enterobacteriaciae
Upper Respiratory Tract		
Pharyngitis	Group A streptococci *(Strep. pyogenes) Arcanobacterium haemolyticus,* Group C streptococci	*Mycoplasma pneumoniae* Mixed anaerobes, *N. gonorrheae, Corynebacterium sp.,*
Otitis media	*Strep. pneumoniae, Moraxella catarrhalis, Haemophilus influenzae,* anaerobes	*Staph. aureus*, Group A *Strep.*
Sinusitis	*Strep. pneumoniae, Moraxella catarrhalis, Haemophilus influenzae,* anaerobes	*Staph. aureus*, Group A *Strep.*
Epiglottitis	*Haemophilus influenzae*	*Strep. pneumoniae, Staph. aureus,* other*Haemophilus*
Otitis externa	*Pseudomonas aeruginosa* (swimmer's ear)	*Staph. aureus*, Group A *Strep.*
Lower respiratory tract		
Bronchitis	*Strep. pneumoniae, Staph. aureus, H. influenzae, Klebsiella pneumoniae, Chlamydia pneumoniae, Mycoplasma pneum.*	*Moraxella catarrhalis, Bordetella pertussis*
Acute pneumonia	*Strep. pneumoniae, Staph. aureus, H. influenzae, Klebsiella pneumoniae, Chlamydia pneumoniae, Mycoplasma pneumoniae, E. coli, Pseudomonas aeruginosa,* mixed anaerobes	*Acinetobacter, Moraxella catarrhalis, Nocardia, N. meningitidis, Mycobacterium tuberculosis,* other *Mycobacterium sp. Eikenella, Francisella, Pasteurella multocida, Ps. pseudomallei, Yersinia pestis, Coxiella burnetti*
Chronic pneumonia	Mixed anaerobes, *Mycobacterium tuberculosis, Nocardia*, other *Mycobacterium sp.*	*Actinomyces, Pseudomonas pseudomallei,*

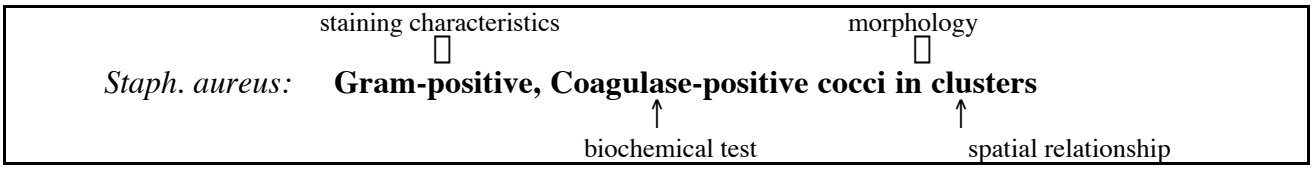
HACEK group = *Haemophilus aphrophilus, Actinobacillus, Cardiobacterium, Coxiella burnetti, Chlamydai psitacci, Eikenella, Kingella*

Organ System	*Common pathogens*	*Uncommon pathogens*
Intra-abdominal		
Spontaneous peritonitis	*E. coli, Klebsiella pneumoniae, Strep. pneumoniae, Enterococcus*	*Staph. aureus,* anaerobes, *N. gonorrheae, Chlamydia trachomatis, M. tuberculosis*
Secondary peritonitis	*E. coli, Bacteroides fragilis,* other enteric anaerobes, *Enterococcus, Ps. aeruginosa*	*Staph. aureus, N. gonorrheae, M. tuberculosis*
Dialysis-associated peritonitis	Coagulase negative *Staph., Staph. aureus, Streptococcus sp., Corynebacterium sp.*	*E. coli, Klebsiella, Enterobacter, Proteus, Ps.*
Intraabdominal abcess	*Bacteroides fragilis* group, *E. coli, Enterococcus sp.*	*Klebsiella, Enterobacter, Proteus, Ps., Staph. aureus*
Urinary Tract		
Cystitis (bladder)	*E. coli, Proteus mirabilis, Klebsiella, Enterobacter, Staph. saprophyticus, Pseudomonas, Enterococcus*	*Staph. aureus, Ureaplasma urealyticum, Corynebacteri-um ureolyticus, Clostridum sp., Bacteroides fragilis*
Pyelonephritis	*E. coli, Proteus mirabilis, Klebsiella, Staph. aureus*	*Enterococcus, Corynebacterium ureolyticus*
Prostatitis	*E. coli, Klebsiella, Enterobacter, Proteus mirabilis, Enterococcus*	*Neisseria gonorrheae*
Genital		
Urethritis	*Neisseria gonorrheae, Chlamydia trachomatis*	*Ureaplasma urealyticum, Mycoplasma genitalum*
Cervicitis	*Neisseria gonorrheae, Chlamydia trachomatis*	*Actinomyces, Mycobacterium tuberculosis*
Bacterial vaginosis (vaginitis)	Synergistic infection with anaerobes *(Mobiluncus, Bacteroides sp., Peptostrepto-coccus)* and possibly *Gardnerella vaginalis*	
Genital ulcers	*Treponema pallidum, Haemophilus ducreyi, Chlamydia trachomatis* (LGV)	
Bone and Joint		
Septic arthritis	*Staph. aureus, N. gonorrheae, Streptococcus sp., Haemophilus influenzae, Borellia burgdorferi*	*Brucella, Nocardia, Mycobacterium sp.*
Osteomyelitis	*Staph. aureus*, Enterobacteriaciae	*M. tuberculosis,* other mycobacterial sp, anaerobes
Prosthesis-associated infection	*Staph. aureus,* coagulase negative Staph., *Streptococcus sp.*	*Peptostreptococcus* misc. aerobic gm - bacilli
Skin & Soft tissue		
Impetigo	*Staph. aureus,* Group A *Streptococcus,*	
Furuncles & Carbuncles (boils)	*Staph. aureus*	
Erysipelas	Group A *Streptococcus*	
Cellulitis	Group A *Streptococcus, Staph. aureus, Haemophilus influenzae*	
Necrotizing cellulitis & fasciitis	Group A *Strep., Clostridium perfingens, B. fragilis, Peptostreptococcus,* other Gm – anaerobes, Enterobacteriaciae, *Ps. aeruginosa*	
Eye		
Conjuctivitis	*Strep. pneumoniae, Staph. aureus,* coagulase neg. *Staph., H. influenzae & aegyptius, N. gonorrheae, Chlamydia trachomatis*	

Table 7 adapted from Murray, Kobayashi, Pfaller, & Rosenthal, *Medical Microbiology, 2nd ed.,* Mosby-Year Book, New York, 1994

IDENTIFICATION AND CLASSIFICATION OF BACTERIA

Bacteria are classified, identified, and described by their staining characteristics, morphology, spatial relationships, and biochemical testing characteristics e.g *Staphyloccocus aureus* can be described in the following way:



A. Microscopic Identification

1. Staining characteristics

   a. Gram's stain - 4 step procedure

      i. apply crystal violet to stain cells blue.
      ii. Iodine solution (mordant) forms crystal violet-iodine complex with cell wall (all cells blue).
      iii. Wash with acetone or alcohol. Decolorizes gram-negative bacteria. Gram positive bacteria remain purple or blue.
      iv. Counterstain with safranin, a red dye that stains gram negatives.

   b. Acid fast stain - useful for identification of Mycobacteria and Nocardia

      i. These organisms aren't stained with safranin in Gram stain.
      ii. Counter stain with carbolfuchsin which binds to mycolic acid in the cell wall of these organisms.

2. Morphology

   a. cocci (spheres) - e.g *Staphyloccocus, Streptococcus, Neisseria*

   b. bacilli (rods) - e.g. Enterobacteriaciae (*E. coli, Proteus*), *Pseudomonas*

      i. spiral shaped rods - *Treponema, Borellia*
      ii. Curved-shape rods - *Vibrio*
      iii. Filamentous rods - *Lactobacillus, Propionobacterium*

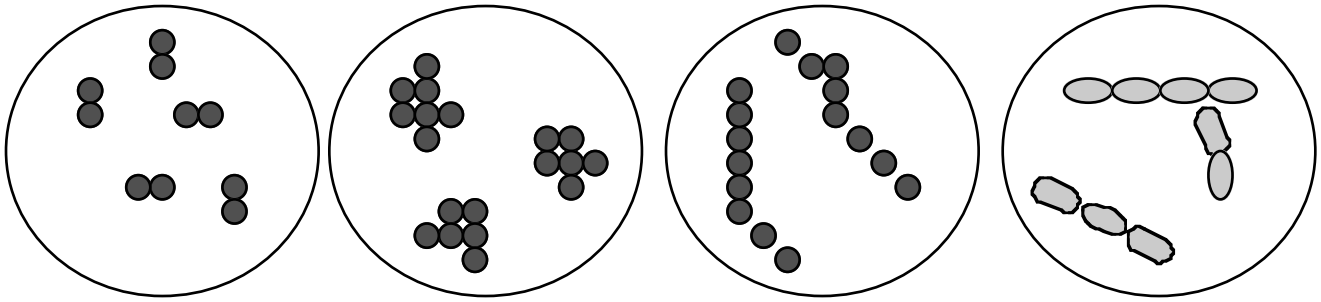
   c. Cocco-bacilli - e.g. *Haemophilus influenzae, Bordetella*

3. Spatial Relationships

a. Chains - *Streptococcus pyogenes,* Group B *Strep., Enterococcus*

b. Pairs - *Streptococococcus pneumoniae* (Gm +), *Neisseria* (Gm –)

c. Clusters (groups) - *Staphylococcus aureus* & *epidermidis*



B. Biochemical Testing (and Morphology)

C. Sensitivity and Selectivity Testing

1. Definitions

MIC = Minimal Inhibitory Concentration. The lowest concentration of antimicrobial agent that will visually inhibit bacterial growth following an overnight incubation (18h) at 35°C of an initial inoculum of 10^4-10^5 colony forming units (CFU) per ml. Usually done in microtiter plates.

MBC = Minimal Bactericidal Concentration. The lowest concentration that kills 99.9% of the bacteria in culture. Requires reculture of MIC incubations that show no growth.

SIT = Serum Inhibitory Titer - Serum from a patient receiving animicrobial therapy is serially diluted & a standard inoculum of bacteria is added to each dilution. The SIT is the greatest dilution that prevents bacterial growth e.g. 1:8 or 1:16 is considered adequate.

SBT = Serum Bactericidal Titer - The SBT is the greatest dilution of serum that will kill 99.9% of the bacteria in the inoculum after re-plating.

Bactericidal = MBC/MIC is ≤4. Indicates that the antibiotic is capable of killing at a concentration near the MIC. Bactericidal antibiotics are aminoglycosides, quinolones, vancomycin, and ß-lactams.

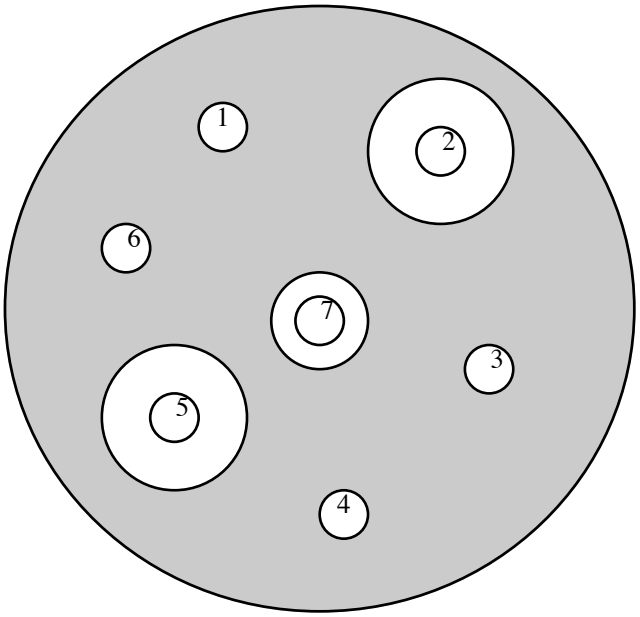
Bacteriostatic = MBC/MIC is between 4 and 32. These antibiotics inhibit growth but require good immune function to eliminate the infecting organism. Examples are chloramphenicol, macrolides, and tetracyclines.

Tolerant = MBC/MIC >32. The bacteria is considered to tolerant to the effects of the antibiotic. *Enterococcus sp*. are often tolerant to aminoglycosides.

2. Sensitivity Testing - Testing of the sensitivity of a patient isolate (a pure culture of the organism isolated from a patient that is responsible for the infection) to a variety of antimicrobial agents.

a. Kirby-Bauer Disk Diffusion Method

1 = Resistant
2 = Sensitive
3 = Resistant
4 = Resistant
5 = Sensitive
6 = Resistant
7 = Intermediate



Paper disks impregnated with various antibiotics are placed on Mueller-Hinton agar plates. The agar is commonly seeded with a set number of CFUs or an even lawn of bacteria is spread on the surface of the plate. The organism is allowed to grow (18 hrs) until the surface of the plate contains contiguous colonies. Antibiotics that inhibit bacterial growth with have a clear zone of inhibition surrounding the plate. Bacteria are usually placed into three groups (Resistant, Intermediate, or Sensitive) depending upon the diameter of the clear zone. When well standardized, the zone diameter can be related to the MIC.

b.) E-Test

A plastic strip impregnated with antibiotic is placed into agar seeded with an even lawn of bacteria. Diffusion into the media provides a continuous concentration gradient that yields a quantitative measurement of the MIC value.

GRAM POSITIVE BACTERIA