Antibiotic susceptibility

**Antibiotic:** natural chemicals produced by bacteria, fungi, actinomycetes, plants or animals, and either inhibits or kills other microbes and/or cells

**Chemotherapeutic agent:** A manmade (synthetic) compound, which inhibits and/or kills other microbes and/or cells

Both work directly on the pathways which microbes use to grow and divide, including: DNA & RNA production, protein and cell wall synthesis, and pathways for other products needed for growth

**Antibiotic Resistance:** Some members of a species become less susceptible to antibiotics levels that previously inhibited growth or killed the ancestral bacteria. *Resistance is acquired.*

Antibiotic use

1) Human treatment and prevention

2) Agriculture (plant and animal) treatment and prevention

3) Growth promoter in food animals
   
   EU began phased out beginning 2005;
   
   US continues to use as food additive

4) Industrialized countries 50%-75% of the annual consumption of antibiotics is due to agricultural use, depends on size of human & animal population

5) Some countries know how much is being used by man and animals
   
   US has only limited idea of how antibiotics are used
Development of Resistance

1. Virtually all pathogens are developing resistance to current therapies:
   - Bacteria, virus, parasites, fungi, yeast, and cancer

2. All therapies will eventually lead to resistance of the pathogen being treated regardless of the source of the therapy

3. Common methods for pathogens development of antimicrobial resistance
   
   a) All infectious agents and cancer develop resistance by mutation of existing genes
   
   b) Bacteria become resistant by acquisition of new genes

Bacteria Genetics

1. Most bacteria have single circular chromosome

2. Some bacteria are naturally transformable: able to take up naked DNA from environment, incorporate the DNA, and express trait. Usually DNA needs to be the same or closely related species
3. **Transduction**: bacteriophage propagated on 1st isolate (donor), packages host DNA and after infecting 2nd isolate (recipient) delivers donor DNA which is incorporated and expressed. Limited to the same or closely related isolate and/or species.

4. **Conjugation**: cell-cell mediated gene transfer that requires the donor to have a conjugative plasmid or conjugative transposon.

Conjugation thought to be the way the majority of clinically relevant antibiotic resistance traits are circulated to bacterial population. Important for transmission of other traits.
History

1. Prior to widespread use of antibiotics in the 1950’s, *Almost all bacteria were not resistant to antibiotics*

2. Bacterial resistance the response to human and agriculture use/misuse World wide

3. Introduction of penicillin in 1945, by 1946 the first penicillin resistant staphylococci were identified

4. Generally associated with transposons/mobile elements/plasmids

![General structure of transposon](image-url)
## Type of Bacterial Antibiotic Resistance

<table>
<thead>
<tr>
<th>Chromosomal mutation</th>
<th>Plasmid/Transposon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usually moderate resistance</td>
<td>Most often high level resistance</td>
</tr>
<tr>
<td>Change in existing structure</td>
<td>Addition of new protein(s)</td>
</tr>
<tr>
<td>Transferred to daughter cells during replication</td>
<td>Transfer to daughter and unrelated isolates, strains, species, genera</td>
</tr>
<tr>
<td>Transfer possible by transformation &amp; transduction</td>
<td>Transfer conjugation, transformation transduction</td>
</tr>
<tr>
<td>Important in some bacteria/ antibiotic combination</td>
<td>Very important clinically</td>
</tr>
<tr>
<td></td>
<td>Most other resistance</td>
</tr>
<tr>
<td></td>
<td>Quinolone, rifampin resistance</td>
</tr>
<tr>
<td></td>
<td>Carry other resistance genes on same unit</td>
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<tr>
<td></td>
<td>Allows for rapid widespread spread of resistance</td>
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<tr>
<td></td>
<td>through bacterial populations and ecosystems; once in bacteria in any environment</td>
</tr>
<tr>
<td></td>
<td>can spread around the world</td>
</tr>
</tbody>
</table>
Methods of Antibiotic Susceptibility Testing

1. Typical bacteria use either disk diffusion, agar dilution, E-test

![Disk diffusion](image1.jpg) ![E-test](image2.jpg)

2. Standardized methods and controls are available- differ in US & EU

3. Breakpoints (susceptible vs resistant) established for typical bacteria

4. Disk diffusion tests developed at the UW Laboratory Medicine 1970’s

5. Similar methods used for fungi, yeast resistance testing, still working on parasites & cancer

6. Viral and *Mycobacterium* spp. use genetic methods or TB use BACTEC 460TB or ESP Culture System II: growth based susceptibility Testing reported within 28 days of receipt of sample

*Salmonella typhimurium*

1975-1982; 20 groups of isolates selected

1 isolate not phage typeable, 14 isolates susceptible

3. Outbreak strains was differentiated from unrelated isolates

Using: phage typing; 67% were typeable; using susceptibility pattern; 44% were typeable- relatively little resistance at this time

**Before 1975 most bacteria carried no antibiotic resistance genes**

Number of resistance genes carried has increased since then

Many parts of the world > 90% of the clinical isolates are multi-drug resistant today
**Advantage of Antibiotic Susceptibility Typing**

1. Simple, fast, reproducible; often done because of clinical relevance
2. Allows comparison by time, location, large number of isolates
3. Data for 30 years
4. Can be done on most bacteria that can be cultured
5. Now being done for fungi, yeast, some viruses, cancer cells, and is being examined for parasites
6. In bacteria in vitro tests usually correlate with in vivo drug/bacteria interaction

**Disadvantages of Antibiotic Susceptibility Typing**

1. Resistance can be lost without antibiotic selective pressure
2. Additional resistance genes can be added to the host
3. Not all genes will express in all bacterial hosts- an issue when looking at out break situation
4. Standards not available for all bacteria
5. Standard susceptibility requires growth of the microbe in the laboratory
6. Standard methods differ between EU and N. America
7. For a useful method, no one group can be > 25% of the isolates examined
8. Varies by type of patient and geography
<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Bacteria target</th>
<th>Bacteria</th>
<th>other</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactams</td>
<td>cell wall synthesis</td>
<td>Gram-positive</td>
<td>Most widely used group of drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gram-negative (newer agents)</td>
<td></td>
</tr>
<tr>
<td>5,000 different compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>protein synthesis</td>
<td>Gram-positive</td>
<td>2nd most used group</td>
</tr>
<tr>
<td></td>
<td>30S ribosome</td>
<td>Gram-negative</td>
<td></td>
</tr>
<tr>
<td>Macrolides</td>
<td>protein synthesis</td>
<td>Gram-positive</td>
<td>Respiratory disease</td>
</tr>
<tr>
<td></td>
<td>30S ribosome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinolones</td>
<td>DNA gyrase</td>
<td>Gram-negative</td>
<td>Urinary tract</td>
</tr>
<tr>
<td></td>
<td>Topoisomerase IV</td>
<td></td>
<td>No Gram-positive mutate too fast</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>cell wall synthesis</td>
<td>Gram-positive</td>
<td>Systemic Disease</td>
</tr>
</tbody>
</table>
Genotypic Methods

1. Look at genetic make-up of the microbe- not expression
2. Some methods need growth of the microbe, others do not
3. All organisms that have DNA or RNA can be tested by at least one of these methods
4. Many of the methods require more experience to perform, need varying amount of expensive equipment
5. Most methods are more expensive to perform than phenotypic methods
6. Less standardization for many of these methods, less standards/kits available
7. **Interpretation** of results may vary between laboratories

Movable elements

1. Conjugative plasmids in enteric bacteria – 1959
2. Conjugative plasmids in *Neisseria, Haemophilus* -1970’s
4. Identify Gram-positive conjugative transposons in Gram-negative bacteria-1990’s
5. Integrons in Gram-negative bacteria and Gram-positive staphylococci-1990’s
Plasmids

1. Small, usually circular, DNA 0.2-10% size of bacterial chromosome

2. Code for nonessential genes, provides bacteria flexibility

3. Can be transferred from cell to cell, strain to strain and between species
   by conjugation, occasionally by transformation

4. Can carry multiple antibiotic resistance genes, virulence factors (toxins,
   adhesion factors), and ability to metabolize different carbon sources, resistance to
   heavy metals

5. Predate antibiotic use

6. Epidemics due to strain carrying a particular plasmid or due to spread
   of plasmid through related species and genera

7. Identification of plasmids now is easy and fast to perform; requires gel
   box and power pack, minimal technical skill

8. Plasmids have variable host range- Gram-negative plasmids do not
   normally function in Gram-positive bacteria

   *E. coli* plasmids share with many related enteric-like bacteria

   *Haemophilus* plasmid shared to related genera
Requirements for Plasmid Typing

1. Some isolates have plasmids others do not
2. Now normally run on plasmid DNA on agarose gels to type
3. Plasmids generally are between 2-300 kb, harder to isolate plasmids over 80 kb because they can break and run with the chromosome
4. Size is determined using plasmids of known weight. Need double stranded standards since linear molecules run different in gel
5. Typing based on counting the number and size of the plasmids each isolate carries
6. Plasmid carriage is not static and over time plasmids can be added or deleted from an isolate
7. Plasmids are usually stable enough for outbreak analysis and for extended analysis-may be lost in storage

Plasmid analysis using Restriction Enzymes

1. Isolate plasmids and cut them into fragments using restriction enzymes, which recognize a particular sequence of the bases (A, T, G, and C). Different enzymes use different sequences
2. The fragments are run on a gel. Determine the relatedness of the plasmids, carry common Antibiotic genes or basic plasmid
3. Can find the same plasmid in other genera. N. gonorrhoeae β-lactamase plasmid originally came from a Haemophilus plasmid, related plasmids were found in H. ducreyi
4. Identify the same antibiotic resistance gene on a variety of different plasmids
5. Related plasmids carry different antibiotic resistance genes
Transposon

1. Defined genetic entities
2. Found in bacteria and eukaryotes including man
3. Have their own genes which allow them to move from one place on a DNA molecule to a new place within the same cell (transposition)
4. Located on plasmids and in chromosome of bacteria
5. Transposons in bacteria can carry antibiotic resistance genes. Do not appear to be associated with resistance in eukaryotes

Conjugative Transposons

1. Defined genetic entities
2. Found in bacteria
3. Have their own genes which allow them to move from one place on a DNA molecule to a new place within the same cell (transposition)
4. Carry their own genes to allow movement from one cell to another by conjugation
5. Located on chromosomes or occasionally associated with plasmids
6. Much larger host range than plasmids- cross most bacterial barriers
Integron

1. DNA element that accumulate and disseminates bacterial genes including: antibiotic resistance, pathogenic traits, and survival against noxious environmental agents like disinfectants

2. Two promoters; 1 expresses the integrase gene, responsible for insertion of resistance genes cassettes at specific sites and the 2nd the antibiotic resistance genes

3. Flanked by 59-base element that allows for recombination, which allows the element to collect new antibiotic resistance genes

4. Located on chromosomes or plasmids

5. Found in Gram-negative bacteria, Gram-positive staphylococci

6. In sequencing genomes, integron structures have been found that do not carry antibiotic resistance genes-predate antibiotic development

7. 2006-Multidrug resistant *Acinetobacter baumannii* 86 kb region with 45 antibiotic resistance genes are clustered- 19 new potential resistance genes
Multi-drug resistant *A. baumannii*

Figure 2. Layout of the Complete AbaR1 Inserted into the AYE Strain ATPase-Encoding Gene
The nomenclature of each ORF is indicated. Each ORF is identified according to EMBL entry CT025832. Colors are used to indicate ORF categories: resistance to antibiotics in red, resistance to heavy metals or antiseptics in blue, transposases in brown, integrases in yellow, and other functions in white. Genes exhibiting a best matching homolog in Pseudomonas are underlined in green, in yellow for Salmonella, in light blue for E. coli, and in turquoise for other bacteria. Complete integrons are indicated by red dashed lines. Transposons are indicated by blue dashed lines. Black dashed lines indicate a gene cluster found in Salmonella genomic island 1.
Outbreak Possibilities

1. Spread of a single strain
2. Spread of a plasmid to a variety of strains and genera
3. Spread of a single or group of antibiotic resistance genes

Advantages of Plasmid Typing

1. Easiest genotypic method to use
2. Minimal equipment, minimal amount of time needed to learn technique and low cost
3. Easiest of genotypic methods for different laboratories to get same answer
4. Variety of kits available for use
5. Quick to perform

Disadvantages of Plasmid Typing

1. Not all species carry plasmids
2. May have large untypeable group because large numbers lack plasmids
3. Plasmids can be lost in storage
4. Large plasmids may be difficult to recover
5. Plasmids may change in size because of loss of >1 antibiotic resistance gene or recombination between 2 different plasmids