MI 822/828

Antibiotics: Overview and Mechanisms of Drug Resistance

The emergence of antibiotic resistant strains of bacteria is a growing problem that is likely to continue.

Infections caused by drug-resistant organisms are more difficult and more expensive to treat than those caused by susceptible organisms. People die from drug resistant organisms when there is no efficacious therapy.

Examples:

Hospital isolates of *Streptococcus pneumoniae*

1987: 4% resistant to penicillin
1997: 48% resistant to penicillin

Resistance to additional antibiotics (1997):

<table>
<thead>
<tr>
<th></th>
<th>All pneumococci</th>
<th>Pen' pneumococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrolides</td>
<td>53%</td>
<td>80%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>15%</td>
<td>51%</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>10%</td>
<td>66%</td>
</tr>
</tbody>
</table>

32 hospitals, 1995:

45% of *Staphylococcus aureus* isolates resistant to methicillin
71.5% of those were also resistant to macrolides
Use of antibiotics selects for resistant bacteria.

- Sometimes, spontaneous mutations just show up when everything else dies. These remain.

The use of antibiotics in animals is probably the greatest contributor to expansion of the pool of resistance genes. BUT excess use of antibiotics is also a problem in humans (shipping, handling, growth, feces, used for soil, has antibiotic resistant bacteria).

Do NOT:
- Antibiotics given for viral infections
- Use of broad-spectrum antibiotic rather than a narrow one
- Prophylactic antibiotics for simple surgical procedures
- Inadequate dosages
- Broad use of antibacterial substances
  - ensure pts finish all prescribed antibiotic (DO THIS!)
  - use inefficient antibacterial substances (soap, etc)

Sources of drug resistant strains of bacteria include:
- Hospitals → many antibiotic resistant strains around
- Animal Feed Lots
- Easily accessible antibiotics in some countries
- Day-care centers → esp. w/ antibiotics for viral infection
  - Antibacterial soap, etc → for like antibiotics, & can get resistant. Need to scrub for 8-10 mins to be effective.

Antibiotic resistance due to:
- a) Mutation of intrinsic genes
- b) Acquision of novel genes

- Some bacteria, fungi, etc produce their own antibiotics for protection & survival
- The attacked bacteria will learn to develop resistant
Mechanisms of Drug Resistance (Biochemical):

1. Inactivation of antibiotic:

   Enzymatic - produce an enzyme that inactivates the drug
   
   e.g. β-lactamase, chloramphenicol acetyltransferase, aminoglycoside phosphatase

   Medical solution: co-administer β-lactam and β-lactamase inhibitor

   Bacteria’s response: produce more β-lactamase to bind inhibitor and still inactivate the β-lactam

   How can bacteria acquire antibiotic-inactivating enzymes?
2. Prevent access to cell:

**Intrinsic barriers**

Gram positive peptidoglycan is a poor barrier to drugs.

Gram negatives have an outer membrane, a more effective barrier, but drugs can enter through pores.

Mycobacteria have additional barrier that makes them intrinsically resistant to many antibiotics. Thick waxy coat impermeable to many things, esp antibiotics.

\[\text{IC, TB}\]

Some mutations can reduce drug uptake.

*mar* mutations of *E. coli* decrease expression of OmpF porin (outer memembrane).

Loss of OprD porin in *Pseudomonas* results in imipenem resistance (OprD is a channel for uptake of basic amino acids (and imipenem)).

Mutant genes can get passed around between bacteria. How?
3. Active efflux systems

Pump antibiotics out of cell, reduce concentration

All require energy (use ATP)

Many different systems, some specific and some efflux multiple drugs

Found in Gram positive and negative organisms

**Genes for efflux systems can also get transferred between bacteria**

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**Examples of active efflux systems in bacteria.**

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Organism</th>
<th>Accessory protein</th>
<th>Gene location</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>OtrB</td>
<td><em>Streptomyces rimosus</em></td>
<td>Chromosome</td>
<td>Oxetracycline</td>
<td></td>
</tr>
<tr>
<td>Tet(L)</td>
<td>Various cocci, <em>Bacillus subtilis</em></td>
<td>Plasmid</td>
<td>Tetetracycline</td>
<td></td>
</tr>
<tr>
<td>Mtr</td>
<td><em>Streptomyces coelicolor</em></td>
<td>Chromeosome</td>
<td>Methyleneomycin</td>
<td></td>
</tr>
<tr>
<td>AcrI</td>
<td><em>S. coelicolor</em></td>
<td>Chromosome</td>
<td>Actinomycin</td>
<td></td>
</tr>
<tr>
<td>TcrnA</td>
<td><em>Streptomyces glaucescens</em></td>
<td>Chromosome</td>
<td>Tetracyclamycin</td>
<td></td>
</tr>
<tr>
<td>NorA</td>
<td><em>Staphylococcus aureus</em></td>
<td>Chromosomal</td>
<td>Fluorquinolones, basic dyes, puromycin, chloramphenicol, tetraphenylphosphonium</td>
<td></td>
</tr>
<tr>
<td>QacA*</td>
<td>S. aureus</td>
<td>Plasmid</td>
<td>Outermost ammonium compounds, basic dyes</td>
<td></td>
</tr>
<tr>
<td>Bmr</td>
<td>B. subtilis</td>
<td>Chromeosome</td>
<td>Basic dyes, chloramphenicol, puromycin, fluoroquinolones</td>
<td></td>
</tr>
<tr>
<td>TetA</td>
<td><em>Escherichia coli</em></td>
<td>None</td>
<td>Tetetracycline</td>
<td></td>
</tr>
<tr>
<td>CmrA</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>None</td>
<td>Chloramphenicol</td>
<td></td>
</tr>
<tr>
<td>Bcr</td>
<td><em>E. coli</em></td>
<td>None</td>
<td>Bicyclomycin</td>
<td></td>
</tr>
<tr>
<td>EnvB</td>
<td><em>E. coli</em></td>
<td>EnvA</td>
<td>CCOP, tetracycline, chloramphenicol</td>
<td></td>
</tr>
<tr>
<td>EnvD</td>
<td><em>E. coli</em></td>
<td>?</td>
<td>CCOP, tetracycline, chloramphenicol</td>
<td></td>
</tr>
<tr>
<td>AcrE(AcrB)*</td>
<td><em>E. coli</em></td>
<td>AcrA</td>
<td>Basic dyes, SDS, tetracycline, chloramphenicol</td>
<td></td>
</tr>
<tr>
<td>EnvC</td>
<td><em>E. coli</em></td>
<td>EnvC</td>
<td>Basic dyes, SDS, tetracycline, chloramphenicol</td>
<td></td>
</tr>
<tr>
<td>MexB</td>
<td><em>P. aeruginosa</em></td>
<td>MexA</td>
<td>Tetracycline, chloramphenicol, fluoroquinolones, β-lactams, pyoverline</td>
<td></td>
</tr>
<tr>
<td>Smr(QacC)*</td>
<td>S. aureus</td>
<td>None</td>
<td>Outermost ammonium compounds, basic dyes</td>
<td></td>
</tr>
<tr>
<td>QacE</td>
<td><em>Klebsiella aerogenes</em></td>
<td>Plasmid</td>
<td>Outermost ammonium compounds, basic dyes</td>
<td></td>
</tr>
<tr>
<td>MsrCe(EnvE)*</td>
<td><em>E. coli</em></td>
<td>?</td>
<td>Methylthiochlorophenylphosphonium, basic dyes</td>
<td></td>
</tr>
<tr>
<td>MexA</td>
<td><em>Staphylococcus epidermatis</em></td>
<td>Plasmid</td>
<td>14- and 15-membered macrodides</td>
<td></td>
</tr>
<tr>
<td>DevA, DevB</td>
<td><em>Streptomyces peucelus</em></td>
<td>Chromosome</td>
<td>Daunorubicin, doxorubicin</td>
<td></td>
</tr>
<tr>
<td>TkC, ThB*</td>
<td><em>Streptomyces lividans</em></td>
<td>Chromosome</td>
<td>Tylosin</td>
<td></td>
</tr>
</tbody>
</table>

*QacA is very similar to QacE. TCCP, carbonyl cyanide m-chlorophenylhydrazone; SOS, sodium dodecylsulfate. Use of names is shown in parentheses. $Alternate names are shown in parentheses. $A study with newly constructed mutants suggests that the original envC mutant contains other, uncharacterized mutations (54).
3. Alter or bypass the drug's target

Examples of altered targets (mutant proteins do not bind antibiotic):

- Streptomycin resistance (protein of 30S ribosomal subunit)
- Rifampin resistance (β-subunit of RNA polymerase)
- Quinolone resistance (GyrA subunit of DNA gyrase)

Example of bypassed target:

Methicillin resistant *Staphylococcus aureus* (MRSA)

β-lactams interact with bacterial proteins, called *penicillin binding proteins* (PBPs), that are involved with *cell wall biosynthesis*

MRSA acquire gene encoding a new *PBP* with a *low affinity to β-lactams*.

β-lactam still *inactivates intrinsic PBPs*, but new *PBP* allows *survival*.

**How can *S. aureus* acquire a new *PBP* gene?**
Vancomycin resistant enterococci → not usually a bad toxin, but is closely related to toxic lact, ie step.

Vancomycin inhibits cell wall synthesis by binding to D-Ala-D-Ala residue on peptidoglycan precursor

Resistant bacteria add D-Ala-D-Lactate instead, so vancomycin can’t bind

Resistance genes are carried on a transposon

Encodes 3 proteins essential for resistance

Encodes 2-component regulatory system sensitive to effects of vancomycin

- Sensory pks
- Transducer-D interacts w/ sensor

1) VanH converts pyruvate to D-Lactate
2) VanA ligates D-Ala and D-Lactate together (cell normally ligates 2 D-Ala together)
3) VanX cleaves any D-Ala-D-Ala formed by native enzyme

* Serves as substrate for Van A

Fig. 2. Schematic representation of peptidoglycan biosynthesis in (a) glycopeptide-susceptible and (b) glycopeptide-resistant cells, showing the activities of VanA, VanH, VanX, and VanY. The incorporation of d-Ala-d-lactate (d-Lac) into the pentapeptide decreases the binding affinity for vancomycin in resistant strains. (Adapted from Ref. 40.) Mur, muramyldipeptide.
Drug resistance in *Mycobacterium tuberculosis*

There are approx. 10,000,000 new cases of TB each year, and approx. 3,000,000 deaths per year due to TB.

Mycobacteria are intrinsically resistant to many antibiotics, so there are very few drugs available for treatment.

Primary drugs: Rifampin, Streptomycin, some Fluoroquinolones (*broad spectrum, mostly resistant anyway*)

TB specific antibiotics: Isoniazid: inhibits mycolic acid synthesis

Ethambutol: inhibits cell wall formation

Pyrazinamide: unknown function

Mutations leading to antibiotic resistant TB:

Resistance to rifampin - *rpoB* (b-subunit of RNA polymerase) → single point mutation

Resistance to streptomycin - *rpsL* (S-12 subunit of ribosome) → 2 point mutations

Resistance to pyrazinamide - *pncA* (nicotinamidase, mutations allow cleavage of PZA) → innate gene for NAD, NADP, etc.

Resistance to ethambutol - *embB* (polymerized saccharides in cell wall)

Resistance to isoniazid - *katG* (catalase-peroxidase [eliminates H$_2$O$_2$], KatG is probably required to activate isoniazid) → mutations in innate genes

*inhA* (involved in mycolic acid synthesis, mutations increase *inhA* expression and result in low-level resistance)