Antibiotic Resistance

Antibiotics are drugs used to kill or block the growth of microbes. They work by targeting cellular processes that are critical for viability. The first antibiotic to be used was penicillin, which blocks peptidoglycan synthesis. It revolutionized medicine, turning previously deadly infectious diseases into easily treated infections. However, within a few years bacteria with resistance to penicillin were observed in patients. We saw that plating *E. coli* on T4 phage allowed only mutants that were resistant to T4 to grow. A similar pattern is observed with antibiotics. A new antibiotic is introduced that kills most bacteria in a population, with the exception of a few resistant mutants. When the antibiotic is used more, susceptible bacteria are killed and resistant mutants survive. Soon, all the bacteria are resistant mutants and the antibiotic is no longer effective.

The most commonly used antibiotics do little harm to patients because they target processes that are unique to bacteria, or they target parts of bacterial enzymes that are different from their human counterparts (see figure below). Peptidoglycan synthesis is a major antibiotic target because peptidoglycan is only found in bacteria. Penicillin and related drugs inhibit many of the enzymes involved in making peptidoglycan. Another important target for antibiotics is protein synthesis. Bacterial ribosomes are different enough from human ribosomes that antibiotics can preferentially inhibit bacterial protein synthesis. A third target is DNA replication. The quinolone class of antibiotics target DNA gyrase, an enzyme that is critical for bacterial chromosome replication. Finally, some antibiotics target enzymes that are essential for growth of bacteria, but are not found in humans. Members of the sulfonamides class of antibiotics inhibit enzymes in the folate biosynthesis pathway.

Antibiotics vary in their ability to kill different kinds of bacteria. In general, Gram-positive bacteria are more sensitive to drugs than Gram-negative bacteria. Gram-positive bacteria lack an outer membrane, so drugs can more readily reach targets inside the cell. Some antibiotics are broad-spectrum and can block the growth of a wide range of bacteria, whereas other antibiotics are narrow-spectrum and only affect a few species of bacteria.

Exposure to antibiotics creates a selective pressure that favors the growth of resistant mutants. There are several major mechanisms that lead to antibiotic resistance. First, mutations in the target molecule can prevent antibiotic binding. Second, bacteria have efflux pumps in their cytoplasmic membranes that can pump drugs out of the cell, so that the drugs don't accumulate to high enough concentrations to do harm. Third, bacteria can make enzymes that degrade or modify the antibiotics, rendering them ineffective. A particularly troubling aspect of antibiotic resistance is that many of the genes that confer resistance are found on mobile genetic elements such as plasmids, so they can be passed from resistant to susceptible bacteria.

Challenge

Pet Microbe- Formulate a hypothesis about the antibiotic susceptibility of your pet microbe. Use the information that you have gathered about your pet to guide your hypothesis. If you have identified your pet, do some research about your pet's species to help you make predictions about its susceptibility to antibiotics.

We will provide the antibiotics listed below. If you would like to test other antibiotics, please let us know and we will try to get them for you.

Antibiotics Provided For Class

Inhibitors of Peptidoglycan Synthesis

Penicillin G - member of the beta-lactam group of antibiotics, inhibits enzymes required for the final stages of peptidoglycan synthesis

Ampicillin - member of the penicillin group of beta-lactam antibiotics, inhibits enzymes required in the final stages of peptidoglycan synthesis

Cephalothin member of cephalosporin class of beta-lactam antibiotics, inhibits enzymes important for the final stages of peptidoglycan synthesis

Vancomycin - member of glycopeptide group of antibiotics, inhibits peptide crosslinking of peptidoglycan by preventing access of transpeptidase enzymes to their substrates.

Inhibitors of Protein Synthesis

Erythromycin - member of macrolide class of antibiotics, blocks translocation of the peptidyl-tRNA from A site to P site of the ribosome

Tetracycline - prevents association of aminoacyl-tRNAs with the ribosome

Doxycycline - member of tetracycline class of antibiotics, prevents association of amino-acyl tRNAs with the ribosome

Minocycline - member of tetracycline class of antibiotics, prevents association of amino-acyl tRNAs with the ribosome

Clindamycin - member of lincosamide family of antibiotics, blocks translocation of ribosome similar to macrolides.

Cloramphenicol - blocks the A site of the ribosome preventing peptide bond formation

Inhibitors of DNA Synthesis

Nalidixic acid - inhibits DNA gyrase

Inhibitors of Enzymatic Pathways

Sulfamethoxazole-Trimethoprim - a mixture of two antibiotics that inhibit different enzymes in the folic acid biosynthetic pathway.

Resources:

Information about antibiotics can be found in any introductory microbiology textbook.

On the web, a good description of antibiotics can be found at the following site.

Antimicrobial agents in the treatment of infectious disease-
http://textbookofbacteriology.net/antimicrobial.html

Antibacterial Drug Targets

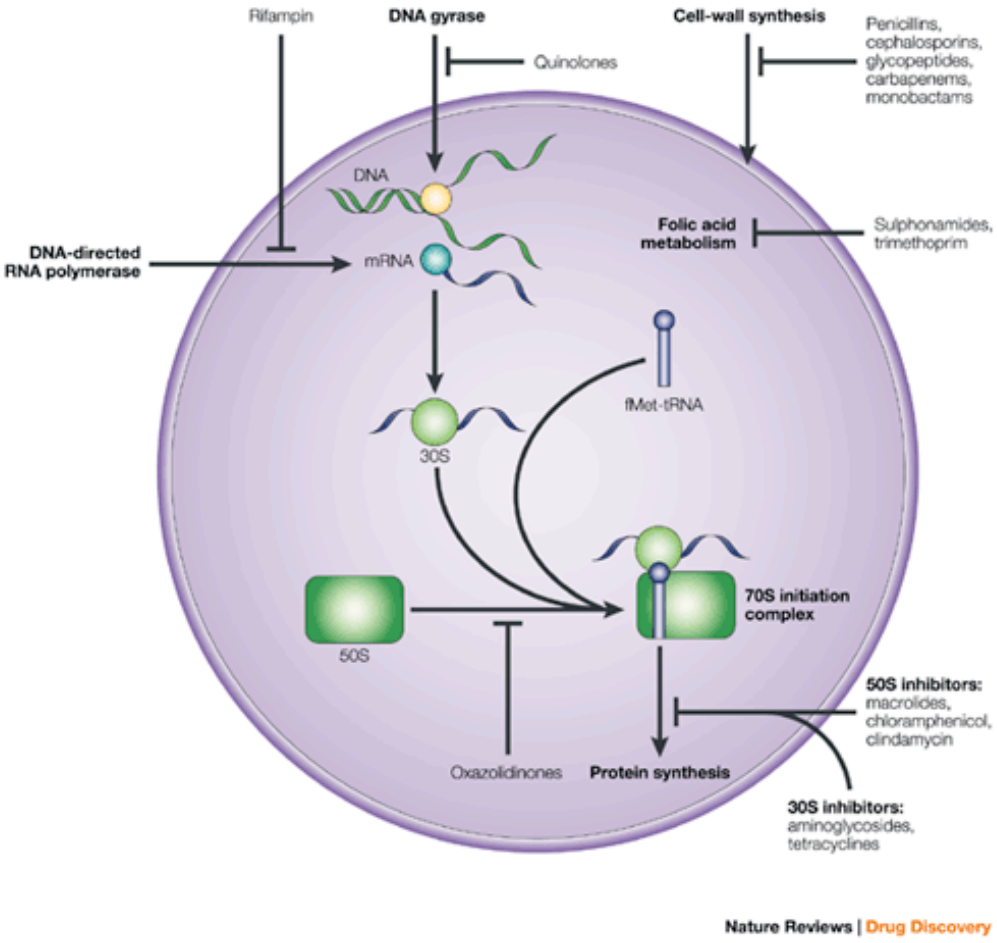
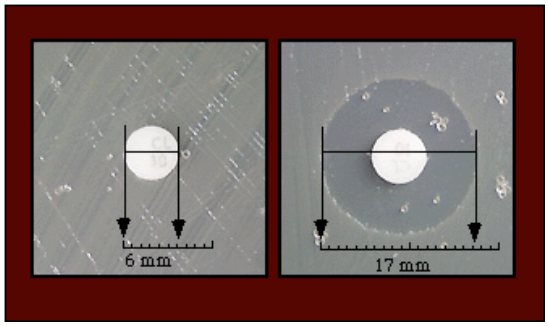


Figure from: Coates A., Hu Y., Bax R. & Page C. The future challenges facing the development of new antimicrobial drugs (2002) Nature Reviews Drug Discovery 1, 895-910

Disc Diffusion Assays

Several different assays are employed to measure antibiotic resistance. We will use the disc diffusion method. In this assay, discs that are impregnated with an antibiotic are placed on a lawn of bacteria. The antibiotic diffuses from the disc across the agar forming a concentration gradient around the disc. The antibiotic concentration decreases with increasing distance from the disc. If the bacteria are susceptible to the antibiotic, they will not grow where the antibiotic concentration is high, and a zone of clearing (with no bacterial growth) will form around the disc. The diameter of the zone of clearing indicates the susceptibility of the bacteria to the antibiotic. The larger the clear zone, the more susceptible the bacteria are to the antibiotic. Bacteria that are completely resistant to the antibiotic will grow right up to the disc and there will be no zone of clearing.



The bacteria are resistant to the antibiotic in the disc on the left and there is not zone of clearing. The bacteria are sensitive to the antibiotic in the disc on the right and a clear zone without bacterial growth is evident around the disc.
Image from: CDC http://www.cdc.gov/std/gonorrhea/lab/diskdiff.htm

Method:

1. Obtain a sterile cotton swab and dip it into your pet microbe culture, thoroughly moistening the cotton swab with the cell suspension.
2. Roll the swab against the sides of the tube to squeeze out excess liquid, although you do not want to let the swab go dry.
3. Use the swab to paint the entire surface of an agar plate.
4. Let dry 15 minutes with the lid slightly ajar until no liquid can be seen.
5. Place antibiotic discs on the plate. Discs should be at least 10 mm from the edge of plate and 30 mm from the center of each other.
6. Incubate plate at appropriate growth temperature
7. Inspect plates from antibiotic-resistance screen for zones of growth inhibition around the discs. Measure the **diameter** (not radius) of each clearing (in **cm**).