Antibiotic Resistance and *P. acnes*

Antibiotics have been in common use since the 1940s and have revolutionized medicine. However, treatment with antibiotics selects for the emergence of resistant mutants. The first antibiotic, penicillin, was introduced in 1943. By 1950, 40% of hospital isolates of *Staphylococcus aureus* were penicillin resistant, and that number increased to 60% by the 1960s[1]. Luckily, new antibiotics have been developed that can be used to treat penicillin-resistant strains. Nevertheless, the cycle of new antibiotics followed by the emergence of resistant strains continues into the present. Unfortunately, the supply of new antibiotics has slowed dramatically. It is now critical that scientists gain a better understanding of the conditions that promote the emergence of resistant bacteria.

Antibiotics are used in many aspects of modern life, from growth promotion in agriculture (from pigs to fruit trees) to medicine. One important question to consider is how or whether the antibiotics that we take to treat illnesses increase the risk of the acquisition of antibiotic resistance. The answer to this question is area of active research. In this class, you have the opportunity to design an experiment to address this important topic. *Propionibacterium acnes* is a resident of your skin microbiome and therefore is potentially exposed to any antibiotics that you take.

In the 1970's the first comprehensive studies of the antibiotic susceptibility of *P. acnes* to commonly used antibiotics were published. *P. acnes* was noted to be susceptible to a wide range of antibiotics. In a work from 1976, 73 *P. acnes* strains were tested against a panel of antibiotics. The strains all showed a common antibiotic susceptibility profile[2]. They were most susceptible to penicillin G, ampicillin, cephalothin, rifampin, erythromycin, clindamycin, and minocycline. Antibiotic treatment to control acne started to be common in the 1980's and has increased substantially since that time. Antibiotics commonly used to treat acne include tetracyline, doxycycline, clindamycin, erythromycin, and minocylcine.

Challenge

> *P. acnes*- Formulate a hypothesis about the antibiotic susceptibility of your *P. acnes* isolate. Consider what properties of the *P. acnes* donor might have affected the antibiotic resistance of his or her *P. acnes*.

1. Chambers, H. F. (2001). The changing epidemiology of Staphylococcus aureus? Emerging Infectious Diseases 7:178-182
2. Hoeffler, U., Ko, H.L., Pulverer, G. (1976). Antimicrobial susceptibility of Propionibacterium acnes and related microbial species Antimicrob Agents Chemother. 10: 387-394.

Key Questions

Processes such as transcription and translation are found in both bacteria and humans. How can inhibitors of these processes act as antibiotics without killing the patient?

You find two colonies growing in the zone of clearing around a disk of ampicillin. If you tested the resistance of these colonies to ampicillin, what would your hypothesis be and why?

Over 80% of the antibiotics used in the US each year are fed to livestock and poultry to increase animal weight and not to treat infections. This high use of antibiotics is thought to be one of the major factors contributing to the rise of antibiotic resistant pathogens. For example, Consumer Reports recently tested pork products (chops, ground pork, and stew meat) for antibiotic resistant bacteria. Around 40% of the bacteria contaminating the meat were resistant to multiple antibiotics. How can the overuse of antibiotics lead to the proliferation of antibiotic resistant strains of bacteria?

Many antibiotics in use today are natural products made by bacteria, fungi, and plants. Why would microbes or plants make antibiotics?

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.

Antibacterial Drug Targets

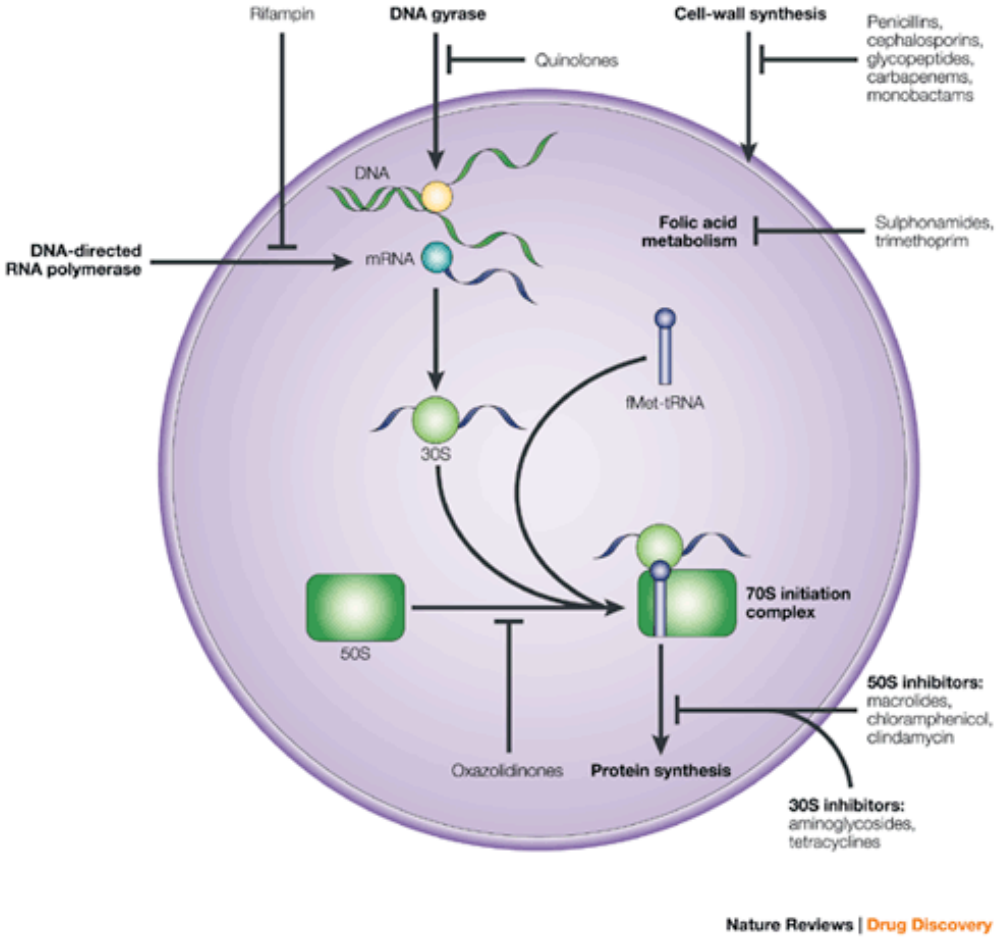
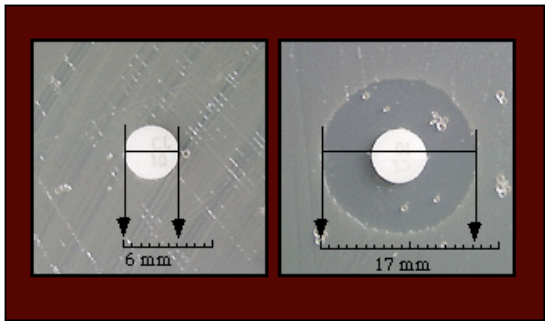


Figure from: Coates A., Hu Y., Bax R. & Page C. The future challenges facing the development of new antimicrobial drugs (2002) Nature Reviews Drug Discovery 1, 895-910

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**).