Evolution/Organic Evolution.
EEES 4150/5150.

The Evolution of Antibiotic Resistance in Bacteria

Purpose:

Soon after the discovery and use of antibiotics for treating infections in humans, a significant rise in antibiotic resistant bacteria was observed. Now, infectious bacteria that were once considered controllable are now difficult to treat due to the prevalence of strains that are either tolerant or resistant to traditional antibiotics. The abuse of antibiotics and poor understanding of how they work are thought to be the primary drivers of the re-emergence of historic diseases and the emergence of new ones. During the past decades an increase in hospital-associated infections due to antibiotic resistant bacteria has been rising steadily. In order to understand how antibiotic resistance has become so prevalent, it is helpful to understand how quickly bacteria can become resistant to antibiotics.

This experiment is designed to show:

1. The capacity of bacteria to evolve rapidly; and

2. Because of this rapid evolution, how quickly biocides and/or antibiotics as treatments to suppress bacterial growth can become ineffective.

Extremely Important:

1. Before the start of the experiment, it is **ABSOLUTELY** necessary for all students to wear gloves.

1. Handle the chemicals and bacteria cultures with caution.

Materials:

Each group of students will receive:

gloves.
5 ml of *E. coli* culture.
2 flame sterilized tweezers.
2 Petri dishes containing:
    triclosan solution (500 μg ml^{-1} in 17% ethanol).
    17% ethanol.
Antibiotic susceptibility testing disks:
    6 blank (no antibiotic)
    3 chloramphenicol disks.

3 cotton swabs.
3 tryptic soy agar media plates.
70% ethanol bottle.
1 ruler (after week 1)

Procedure:

1. Spray 70% ethanol on the bench and wipe clean.

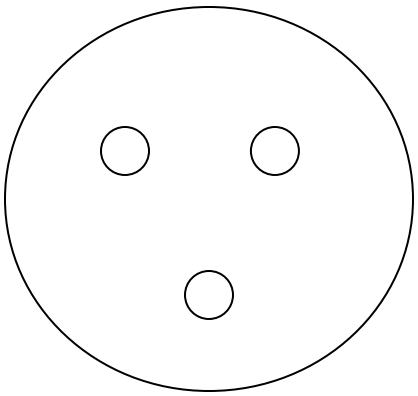
2. Label the bottom of each Petri dish that contains growth media with either "triclosan", "17% ethanol" or "chloramphenicol", the date and the name of your group leader.

3. Shake the *E. coli* culture to mix, and then dip the cotton head of the swab into the culture and swirl for few seconds.

4. Streak one of the TSA plates with the swab by passing the swab over the surface of the agar plate. Turn the plate 90^{o} and re-swab. The idea here is to completely cover the plate with *E. coli*, so that after incubation and growth, a lawn of bacteria is formed. Repeat for the remaining two Petri dishes.

5. Using tweezers, transfer 3 blank antibiotic susceptibility disks to the Petri dish containing triclosan solution. Submerge for 10 seconds.

6. Using the same tweezers, blot-dry the disks you just submerged on a piece of paper towel for five seconds per side. Evenly distribute the disks onto the media in the appropriately labeled Petri dish, as shown in the figure below.



6. Using a different set of tweezers, transfer 3 blank antibiotic susceptibility disks to the Petri dish containing 17% ethanol. Submerge for 10 seconds. Blot dry and apply to the appropriate Petri dish as described above.

7. Using the tweezers, press lightly on the disks to make sure that each is in contact with the surface of the agar (you do not want them to penetrate the media, so don't push too hard).

8. Repeat the steps above using the chloramphenicol disks, however these disks can be directly applied to the media. **They should not be soaked in any solution**.

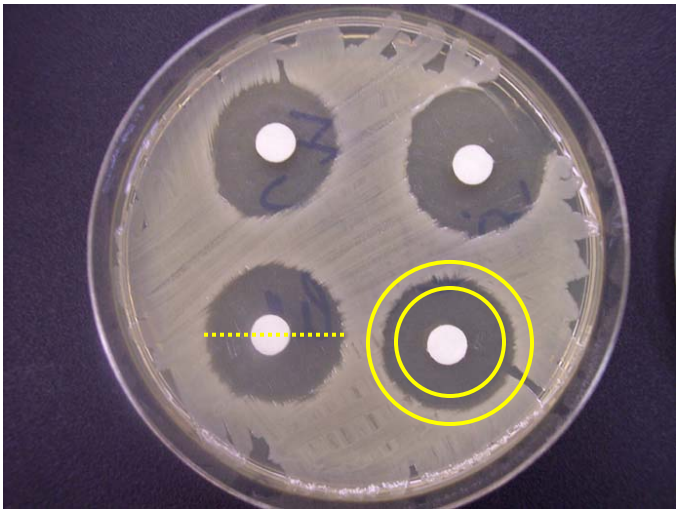
9. Organize the plates and hand them over to the assistant, who will place them in an incubator at 37 ºC.

10. During the next lab session the results will be interpreted.

Next time:

1. Spray 70% ethanol on the bench and wipe clean.

2. For each Petri dish and susceptibility disk, determine the size of the zone of growth inhibition by measuring the diameter (in mm) of the circular zone where no growth is occurring. An example of a susceptibility assay is shown below, including a zone of inhibition surrounding four antibiotic susceptibility disks. The dotted line shows the correct location to measure.



3. Calculate the average and standard error of the zone diameter for each treatment.

4. For next week, repeat the protocol beginning at step 2, except instead of using *E. coli* from a liquid culture, you will carefully collect *E. coli* from your Petri dishes. Using a sterile swab, collect *E. coli* from the margin of growth (indicated by the area between the double circle in the figure above). Transfer

these *E. coli* to fresh growth media as described above, keeping in mind that you want to completely cover the media surface with *E. coli.*

5. Continue with step 5, above.