Overgrowth of Fungi as a "Side-effect" of Antibiotic Use

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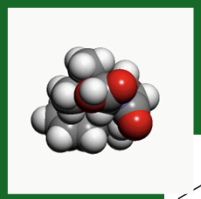
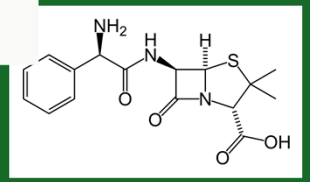
Abstract
Students in the Biological Evolution course at St. Francis College noticed that the Luria –Bertani (LB)agar plates with and without ampicillin had become contaminated with mold after they were made and stored for two weeks in the refrigerator. We were supposed to use these plates for an antibiotic-selection experiment for *E.coli* but switched to an examination of the "contamination" instead. The LB plates plus ampicillin had more mold than the control LB plates, which puzzled us, until we read that this "overgrowth" was a side effect of the antibiotic. Ten white and 87 reddish brown colonies were found on the LB control plates, whereas 29 white and 112 reddish brown colonies were found on the LB + ampicillin plates. ($p < 0.01$ with a Chi-squared analysis.) The white colony size in mm average was slightly larger in LB control plates versus LB + amp plates (18 and 12 respectively) , but the reddish brown colony size average was approximately 7 mm in both. This experiment represents a simulation of what can occur in the body as a result of antibiotic use.

Introduction
My students in the Biological Evolution course were all set to plate out bacteria on Luria Broth (LB) control plates and LB plates to which the antibiotic ampicillin had been added. But, unfortunately, our refrigerated plates (for two weeks) had mold growing on them. After a perfunctory glance, I (KN) noticed that the LB + amp plates appeared to have more fungus growing on them than did the control plates. Upon further research, we found that administering ampicillin as an antibiotic can cause an overgrowth of fungus. I (KN) have had experience in this area with my child who was given amoxicillin for an ear infection. She developed a yeast infection in the diaper area, and we next gave her Nystatin, an anti-fungal. Interestingly, once the fungi were inactivated, a bacterium that causes impetigo came into play. This personal experience revealed to me first-hand how the use of an antibiotic can "tip" the balance of flora in our microbiomes.





Student Outline

Obtain a bottle of Luria Broth Agar and put in a water bath until it is melted. Alternatively, your instructor may have already done this.

When the agar has cooled to 50o C, ampicillin can be added.

When the agar has cooled enough so as not to melt the plastic petri plates (the bottle should be comfortable to handle) pour your plates.

Pour plates with a bottle of LB alone as a control.

Leave the cover off of the plates for one hour.

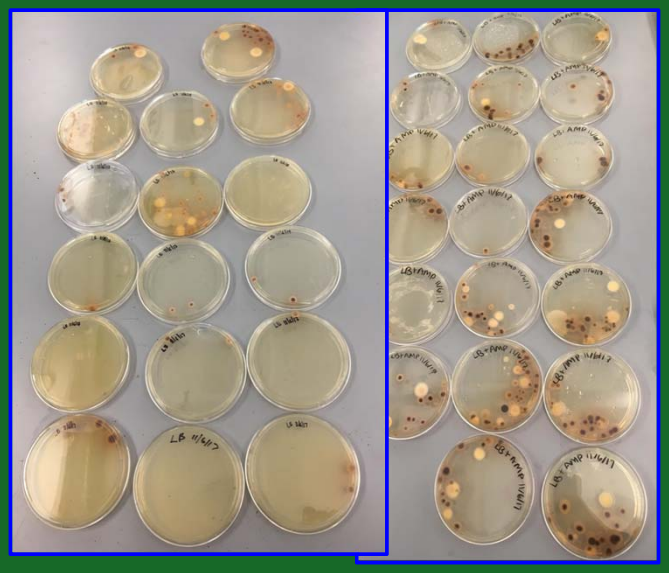
Cover the plates and refrigerate for two weeks.

Observe, measure and count colonies. Try to categorize by color. When we did this experiment, we had predominantly two types of colonies—white and reddish-brown. On two out of 34 plates, we observed two bright orange yeasts.

Put your data in Excel spread sheet---a sample is shown.
Calculate the number of counts of each type of algae on each plate type. Perform a X2 test to see if there is a significant difference between the types of counts.

Recipe
LB (Luria-Bertani) agar medium (from Cold Spring Harbor Protocols)
Reagent — Amount to add
H_2O — 950 mL
Tryptone — 10 g
NaCl — 10 g
Yeast extract — 5 g
15g Agar
Combine the reagents and shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH (~0.2 mL). Adjust the final volume of the solution to 1 L with H_2O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle.

Results



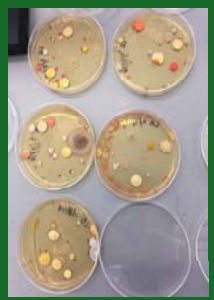
17 plates	# of colonies		diameter of colonies (mm)	
	white	red/brown	white	red/brown
LB control				
	10	87	18.45	7.69
LB + amp				
	29**	112**	12.86	7.60

Discussion

We were able to turn what we thought was a "failed" experiment into something that made us think more deeply and learn additional information about antibiotics, antibiotic resistance and possible side effects of antibiotics. We saw a statistical difference in number of molds that grew on LB plates versus LB plates that had been supplemented with ampicillin. It has been shown (and personally experienced by an author) that excess fungal growth can be a side effect of antibiotics. We feel that this experiment shows that this excess fungal growth on LB + amp plates could be analogous to what happens in our own bodies.

Additional inquiry-based experiments
This spring (2018), a group of students in the BIO 1202 General Biology II class decided to try some variations on this experiment. Their experiments were dictated partially by what was available in the teaching labs. The students tried opening Sabouraud dextrose agar (a medium supports fungal growth) plates for an hour, and then incubated plates at various temperatures (30°C, 4°C (refrigerator) and 21°C. (room temperature). Only a few moles were growing on the plates after a week. The students repeated the experiment with LB with and without kanamycin, but left the covers off the plates overnight (10 hours). The students observed a variety of fungal growth (see examples below) but did not observe any significant differences between types of plates supplemented with kanamycin and those without.

This summer (2018) the BIO 3303 Microbiology course will repeat the original experiment (LB control plates and LB + amp) and will leave the plates uncovered for three hours. We will see if they will get results similar to those in Fall 2017.



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