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A Simple Experiment that Reveals Overgrowth of Fungi as a "Side-Effect" of Antibiotic Use

Kathleen A. Nolan, Victoria Ruiz, Allen J. Burdowski, Kristen Casares and Onika Brown

St. Francis College, Biology, Health Promotion, and Health Care Management Dept., 180 Remsen St., Brooklyn NY 11201 USA
(**knolan@sfc.edu**; **vruiz@sfc.edu**; **aburdowski@sfc.edu**)

Students in the Biological Evolution course at St. Francis College noticed that the Luria–Bertani (LB) agar plates with and without ampicillin (amp) 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.

Keywords: microbiology, fungi, antibiotics

Link to Original Poster: **http://www.ableweb.org/volumes/vol-40/poster?art=69**

Introduction

Students in the Biological Evolution course were all set to plate out bacteria on Luria-Bertani (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, Nolan noticed that the LB + amp plates appeared to have more fungi 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. Nolan has had experience in this area with her baby who was given amoxicillin for an ear infection. The baby developed a yeast infection in the diaper area, to which was applied Nystatin, an anti-fungal. Interestingly, once the yeast disappeared, a bacterium that causes impetigo produced an additional rash that appeared to be quite different from the yeast rash. This personal experience revealed first-hand how the use of an antibiotic can "tip" the balance of flora in our microbiomes.

A side effect of the use of ampicillin is fungal growth (Voychuk et al., 2010). Yu-Kyong and Young (2017) note that ampicillin can activate phosphorylation (and thus, growth) in yeast. One intriguing side effect of the use of antibiotics, which has also been associated with other conditions such as smoking or a dry mouth, has been Black Hairy Tongue (Thompson and Kessler, 2010), which has not been fully characterized (Figure 1).

The causes are either uncertain or varied; one could be a "chromogenic-producing microorganism". Ferreira et al. (2017) collected data from studies of how 68 antibiotics can affect the human microbiome.

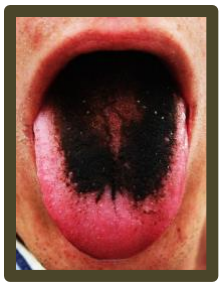

Figure 1. Black Hairy Tongue (Creative Commons)

Zimmerman et al. (2017) remark that overprescribing antibiotics may further contribute to side effects of these antibiotics. Anghel et al. (2013) point out that all organisms have natural defense molecules called cytotoxic peptides, which could help explain the growth of the fungi in people with opportunistic infections. Moreover, Ferreira and Santos (2017) note in a review that heteroresistance can develop in fungi, which is a differential resistance of fungi to antifungals, which is a growing problem. Rojo et al. (2017) invite us to explore the human microbiome and all its intrigues and complications. This study would give us preliminary information about the fungi that are present in the air around us, and how increased fungal growth in the presence of an antibiotic against bacteria might aid us in making conclusions about what might be happening in our own bodies.

We have conducted a preliminary experiment in which we have exposed LB plates to air and then refrigerated them. We found that there was a significantly greater difference in mold growth on plates on LB plates plus ampicillin than on LB plates alone. 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. See Fig. 2.

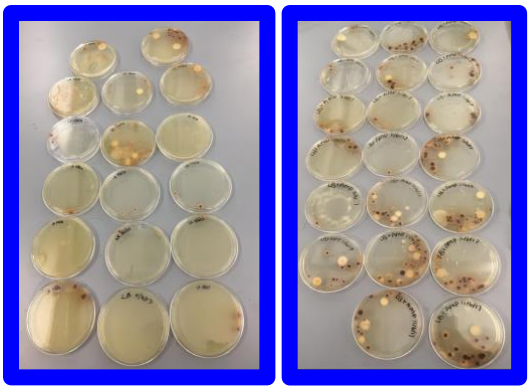

Figure 2. LB alone plates on left and LB + amp plates on right after two weeks in refrigerator. Many of the plates were contaminated by molds.

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, 10°C (refrigerator) and 22°C. (room temperature). Only a few molds were growing on the plates after a week. The students repeated the experiment with LB with and without amp, but left the covers off the plates overnight (10 hours). The students observed a variety of fungal growth that was again different in LB plates versus those supplemented with ampicillin (Fig. 3).

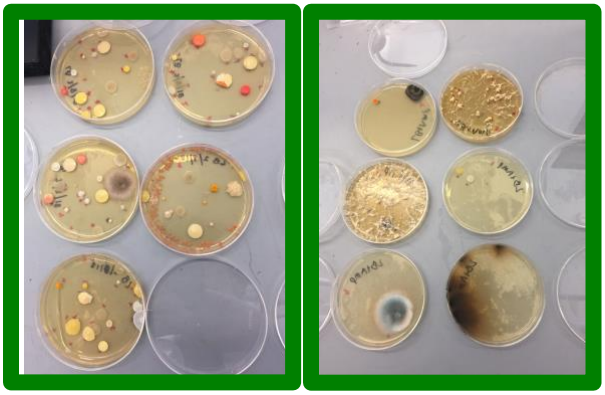

Figure 3. LB plates alone (left) LB – amp (right)

Student Outline

Objectives

- Learn microbiological techniques
- Design an experiment
- Test hypotheses
- Make tables and analyze data

(Read Introduction above)

1. Obtain a bottle of Luria Broth Agar and microwave it until it is melted. (Alternatively, your instructor may have already done this and placed the bottle in a 50° C water bath.)

2. When the agar has cooled to 50° C, ampicillin can be added. (A hotter temperature destroys the antibiotic.)

3. 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.

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

5. Leave the cover off of the plates for one to three hours.

6. Cover the plates and either refrigerate for two weeks or leave at RT for up to two weeks (your instructor will decide which treatment you should use).You may also choose to incubate all plates, after all treatments at 30°C, which is the optimal growth temperature for many fungi.

7. 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.

8. Put your data in Excel spread sheet.
   Calculate the number of counts of each type of molds or yeasts on each plate type. Perform a X^2 test to see if there is a significant difference between the types of counts. Also state the range in size of each color of colony that you observe.

Discussion

Sangamwar (2008) point out how fungal infections have increased dramatically in these times, and that they are hard to treat since they are eukaryotes, and that they are often difficult to diagnose. In the future we hope to gain skills in tools used in identifying fungi through classic tests, and by characterizing them through DNA analysis. An experiment such as this could be the beginning of training how to eradicate future fungal infections. For example, Adimi et al. (2013) tested ten antifungals against 320 dermatophyte (ring-worm causing) strains of fungus.

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.

Materials

We performed this experiment with Luria–Bertani agar and ampicillin; did not leave plates open; and refrigerated the plates for two weeks.

We used a concentration of 100 µL of a 100 mg/mL ampicillin stock (1000X stock) to 100 mL of LB.

It could also be done with Sabouraud's agar and a variety of antibiotics.

Rulers and computers for entering the data

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
Agar	15 g

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.

Notes for the Instructor

If time permits, the students can streak out the LB alone and LB + amp plates with *E. coli* to make sure that the ampicillin is working. Alternatively, this could be a demonstration. The amp should kill the *E.coli.* See Fig. 4.

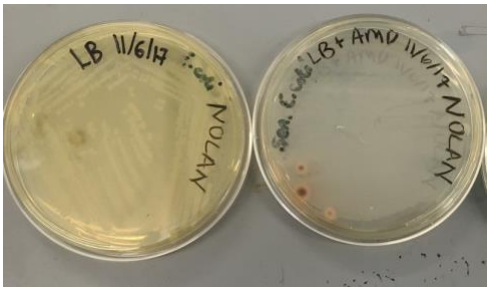

Figure 4. Plate on left shows growth of *E.coli* in LB alone, but no growth on the LB = amp plate on the right. Note red mold contamination of plate on right.

This experiment can be conducted as an inquiry-based experiment in which students pick the media, the type of antibiotic, and the conditions, when possible. When students were asked to design other treatments for the experiment, one student produced this table:

Treatment	LB alone RT	LB + amp	LB alone 10°C	LB + amp	LB alone 30°C	LB + amp
Closed						
Open 3 hrs.						

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About the Authors

Kathleen A. Nolan, Ph.D. is a professor of biology and Chair of the Biology and Health Sciences Department at St. Francis College. She has been a long- time ABLE member and has presented numerous major and mini-workshops at ABLE conferences. She is interested in a wide variety of topics, including fish population genetics, animal vocalizations, and biology laboratory education.

Victoria Ruiz, Ph.D. is in her second year at St. Francis College as an assistant professor of biology. She received her Ph.D. from Brown University, and has conducted post-doctoral work in microbiology at New York University. She teaches microbiology, anatomy and physiology, and general biology. She is very much interested in teaching and learning.

Allen J. Burdowski, Ph.D. is the Dean of Science and Health at St. Francis College. He teaches a variety of courses including Pharmacology and Animal Physiology. He is very interested in different methods of instruction for STEM majors.

Kristen Casares and Onika Brown were biology majors at St. Francis College when they helped conduct this project. They are now graduates of St. Francis College.

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