

Appendix 1: Week 1 activities

Before class: As you prepare for your first day, we have your first assignment. Please watch a couple videos on a scientist/researcher/keynote speaker and select one that you would like to share with your discussion section groups. Be prepared to talk about:

1. The scientist's name
2. Their job title
3. What inspired them to pursue their job/research (this could be a life experience, a specific scientific question, etc.)
4. Challenges they faced plus how they overcame them
5. The impact of their work

Think carefully about the application of their research and how the type of research they do impacts their mode of communication with the world (i.e. is it through publication of a manuscript? development and launch of a new technology? impacting a long-standing policy? etc.)

Here are a couple websites to guide your search:

<https://www.youtube.com/sacnas>

<https://www.ibiology.org/stories/profiles/>

In class discussion:

Short lecture: During this session students are provided information on the necessary scientific background for them to begin their research projects.

Small groups: Have the students share about the scientist of their choosing from the homework above. Below are potential questions/topics for discussion:

- Why did they choose this scientist?
- Can they identify with the scientist they chose?
- How would the world be without their selected scientist's discovery?
- Have they heard of the other students' selected scientists?
- Do they plan to become scientists?
- What are their passions? (passions can become jobs/careers)
- What is their experience level in science?
- What kind of STEM do they like? Physics? Engineering? Chemistry?
- Have they ever done the scientific method but didn't know?
- What goals do they have with this program?
- Why did they choose to participate?
- What are their thoughts on the activities we did today?

Post-class Homework:

Article:

Gehad, Y., & Springel, M. (2020). *Characterization of antibacterial properties of common spices*. Journal of Emerging Investigators. <https://emerginginvestigators.org/articles/characterization-of-antibacterial-properties-of-common-spices/pdf>

Complete the following handout using the paper cited above:



Journal of Emerging Investigators

Where students become scientists

Introduction to Scientific Primary Literature

Much learning is done through reading textbooks. But where does the information in textbooks come from? This handout and accompanying paper will help you learn more about how information makes its way to your textbook.

At universities, and other research institutes, there are many scientists performing research. When an interesting discovery is made, it must be shared with other scientists working on similar research. Scientists also have a responsibility to share their findings with the public.

The primary way to share results is by publishing a paper in a research journal, similar to the paper we will be looking at today. When a paper is submitted for publication, it is sent out to several other experts in the field. These reviewers assess the rigor of the research and provide feedback to the authors. This process is called peer review. Once the authors of the manuscript address the suggestions of the reviewers, their paper is published and made available for others to read.

When an interesting discovery is made, other scientists may try to reproduce the findings and either confirm or refine the initial results. Over time, as the initial discovery is supported by further evidence (passes the “test of time”), it makes its way into your textbook!

The paper for today was published in the Journal of Emerging Investigators (JEI), with research performed by a middle or high school student. JEI accepts submissions from any middle or high school student interested in publishing a scientific manuscript. Just as in other professional journals, papers submitted to JEI go through a peer review process. Once the paper is resubmitted with the changes recommended by the reviewers, it is published to the JEI website (www.emerginginvestigators.org/) for anyone to read.

Paper overview:

Every published paper, regardless of the journal in which it was published, has a few key components. These components help identify who did the research, what the research was about, where and when and how it was performed, and why the research is impactful. Think about these questions as you complete this worksheet:

Title of the manuscript

Names of the authors

Schools where the scientists performed the work

Date when article was received

Date when article was published

Why might bacterial infection be a problem?

How do bacteria develop resistance to antibiotics?

What literature inspired the authors to test spices in this paper? Be specific with any titles

What terms did the authors decide to define and where are these definitions in the manuscript? Why do you think it is important to define terms?

What is the main question that the authors are trying to answer?

Where is the hypothesis and what is the hypothesis? Why is it stated in this part of the paper?

Why is there a concluding sentence in the introduction? Why do you think it's placed there?
How did they define negative control? How did they define positive control?

What are the units used to measure colony size and why?

What do the bars in Figure 4B represent?

Why do you think the authors include pictures in addition to bar graph depictions of data?

Why was it important to test varying concentrations of spices (the authors performed dose titrations of spices) as opposed to testing just one concentration of spice?

How did the negative and positive controls turn out? Were these control results as expected?

Were the authors explicit about their hypothesis being true or false and if so, where is it found? Why do you think it is placed in this location?

The authors measure optical density in the manuscript. What are authors testing and how does it reflect bacterial growth? Does this method have any advantages or disadvantages?

"We grinded each of the 11 spices to a fine consistency, mixed them into LB agar at 5% concentration, and made LB plates using this agar. Equal amounts of E. coli were spread on each plate, three replicates of which were incubated overnight at 37°C." Where does this sentence look like it should be placed? In which section is it actually placed? Why do you think it has been placed there?

Were the results of the experiment expected? How did the authors explain their findings in the context of what they hypothesized?

Where do the authors refer to previous studies or other research? Why do you think these references are placed in these locations?

What are the pitfalls of the experiment and where are they located in the manuscript? If you were an author of this paper, what follow-up experiments would you want to perform to continue investigations?

What is the significance of this research and where is it found in the manuscript?

What is the purpose of the summary (go back to the first paragraph of the manuscript, written in blue)? What content or information does the summary include? Describe the structure.

Appendix 2: Week 2 activities

Before class: Complete homework assignment from week 1.

In class discussion:

Facilitators discuss student answers from the HW worksheet. Potential questions or prompts for group discussion:

- Knowns and unknowns regarding bacteria and their living environments? (Background)
- What is the scientific question the authors are trying to address? What's their hypothesis?
- Why is answering this question significant?
- Review the structure of an introduction section
- Why did the authors include pictures in addition to the graphs?
- Where and what is the hypothesis?
- Why is the hypothesis given in this part of the paper?
- What question are the authors trying to answer?
- How do bacteria develop resistance to antibiotics?
- What literature inspired the authors to test the spices in this paper? (ref6)
- (Must define terms in a manuscript) What terms did the authors decide to define and where are these definitions in the manuscript? Distinction between gram-positive and negative bacteria? OR why is it important to define terms?
- How did they define neg control? Pos control?
- Why are the units for the colony mm^2 ?
- What do the bars in 4B represent?
- Why is varying concentrations of spice important (dose titration) as opposed to just one concentration?
- Were the authors explicit about their hypothesis being true or false and where is it found? Why is it placed there?
- Are there any sentences in the manuscript that you think should have had a reference but didn't? Or vice versa, there was but didn't need a ref?
- What were your thoughts on the timeline that the manuscript was received, accepted, and published? What do those terms mean?
- What do they think optical density refers to? How does it reflect bacteria growth? Why do they use this method?
- After reading this paper, what followup experiments would you perform to follow up investigations? Is there any data that you would like to see to be more persuaded by conclusions?
- Were the results of the experiment expected? How did the authors explain their findings in the context of what they hypothesized?
- Where do the authors refer to previous studies or other research? Why do you think these references are placed in these locations?
- (Even though it seems like a methods section sentence, why is this in the results section? Answer: It needs to be contextualized). "Equal amounts of E. coli were spread on each plate, three replicates of which were incubated overnight at 37°C." OR "we grinded each of the 11 spices to a fine consistency, mixed them into LB agar at 5% concentration, and made LB plates using this agar. Equal amounts of E. coli were spread on each plate, three replicates of which were incubated overnight at 37°C."
- What are the pitfalls of the experiment? Where are they located?

- Why is there a concluding sentence in the introduction? Why do you think it's placed there?

Post-Class Homework:

Students will contribute to building a group glossary, defining terms which are relevant to the experiment topic and can be referenced for writing their manuscripts. An example group glossary is below:

JEI group glossary (Sample answers from students)

Add your definition to any word.

If a word already has defining information, but you have something else to add, go ahead and add it!

If there's a word you'd like defined, add the word!

Link to this doc for your copying-and-pasting convenience: <https://tinyurl.com/groupglossary>

Colour (pigmentation) – for example, white, buff, red, purple, etc.

Agar - It is commonly used in the laboratory to help feed and grow bacteria and other microbes. It acts as a culture that provides nutrients and a place for these microbes to go, but since it is indigestible to the microorganisms they cannot eat or destroy it.

Amino acid - An amino acid is the fundamental molecule that serves as the building block for all proteins. Amino Acids are converted to proteins in ribosomes, Sections of DNA code for different amino acids.

Assay - the testing of a material to determine its ingredient or quality

Bacterial Culture - a method that allows the reproduction of bacterial cells under controlled laboratory conditions.

Colony - A group of bacteria that (theoretically) forms when one, viable cell divides/reproduces to make genetically identical copies (except for when daughter cells have mutations, which are rare). However, not all colonies form from only one cell.

Colony Forming Unit (CFU) - A unit used to estimate the number of bacteria or fungal cells in a sample.

Confluence - When bacterial colonies merge and overcrowd/cover either the entire surface [of the bacteria plate] or a portion of it. Confluence can be measured as a percentage; for instance, 75% confluence means about three-quarters of the surface is covered.

Countable range - The suitable colony counting range is 25-250. It is important because it avoids any overcrowding errors.

Edge effect- colonies that are difficult to count, because they are on the edge of a petri dish.

Homogeneous - consistency throughout a substance.

Kirby-bauer disc - N/A

Latency - The time between the development of a disease or exposure to a pathogen, chemical, or radiation and when symptoms first became apparent or when the disease becomes infectious.

Morphology - The study of the forms of things or the study of the shapes and arrangement of parts of organisms in order to determine their development.

Pathogen - anything that makes you sick.

Serial dilution - Is a series of sequential dilutions that are performed to convert a dense solution into a more usable concentration.

Spontaneous Generation - a hypothetical organic phenomenon in which living organisms come to exist from nonliving matter

Oblong Colony - aka a merged colony; is formed when two colonies are close enough to each other to merge together

Translation - The process by which the cell makes proteins using the genetic information carried in messenger RNA.

Turbidity - The state or quality of being clouded or opaque, usually because of suspended matter or stirred-up sediment

Watershed algorithm - based on extracting sure background and foreground and then using markers will make watershed run and detect the exact boundaries.

Bonus question: What does the turbidity of a solution tell you about microbial growth?

Resistance - The natural or genetic ability of an organism to avoid or repel attacks by biotic agents.

Opacity – for example, transparent (clear), opaque, translucent (like looking through frosted glass), etc.

Density - Measure of how compact a substance is.

Minimum inhibitory concentration - The lowest concentrations of an antimycobacterial that will inhibit the visible growth of a microorganism after overnight incubation and minimum bactericidal concentrations.

Sterile - Free from bacteria or other living microorganisms or totally clean.

Incubation - The maintenance of uniform conditions of temperature and humidity to ensure the development of certain experimental organisms, especially bacteria.

Atypical - Uncommon, Unusual, Irregular.

Desiccation - Remove moisture, dehydration; represents the drying out of a cell in biological terms.

Enumeration - The process of determining the number of bacteria in a given sample.

Spreader - Colonies that appear to "spread" across the plate.

Appendix 3: Week 3 activities

Before class: Complete homework assignment from week 2.

In class discussion:

Facilitators will review the homework completed by the students. Then facilitate the group discussion with the following prompts:

1. Discuss how students counted the bacterial colonies on each petri dish and why they chose to count in that manner (answers can be quantitative or descriptive).
 - If they counted a specific number, ask how many and why.
 - If there was a lawn of bacteria, ask the students why they think there could have been a lawn. Describe that this can happen to their own swabbing experiments.
 - Ask how they can prevent a lawn of bacteria for their own experiments?
2. Open up the group glossary and ask the students which terms they defined and what the definitions were. Ask if there were any terms the students were unsure about.
3. Start the experimental design template (below) with group members. Students will start experiments following the next session based on this experimental design.

Post-class homework:

- Complete experimental design and hypothesis building with groups
- Fill out worksheet (below) in preparation for learning about Methods

Experimental Design Template

Research question:

Known background:

Why we care: (no wrong answers)

Hypothesis:

General hypothesis-based predictions (*don't need experimental details*):

Specific hypothesis-based predictions (*here's where experimental specifics go*)(*include where you will swab, what will be your antimicrobial reagents, why you are selecting these locations/reagents, what your predictions are, and why you are making these predictions*):

Negative control(s):

Positive control(s):

Materials:

Protocol:

Possible results and their interpretations:



Introduction to Materials and Methods section:

Recipes for some dishes, such as stew, can be followed with a bit of culinary leeway, while those for other dishes, such as soufflés, must be followed with a bit more care if the cook hopes to produce something esculent and attractive. Woe is the baker who omits a critical ingredient from the cake recipe transcribed for an admirer! **These culinary caveats are relevant because the Materials and Methods section of a hypothesis-testing article often is compared to a recipe. Like the intricate soufflé, the Materials and Methods “recipe” must be described precisely as followed.** This precise description assures readers that the correct supplies and processes were used to answer the research question posed in the Introduction of the article and ensures that other researchers have the information necessary to replicate the study. The bulk of the Materials and Methods section is, therefore, **a detailed description of all materials and methods used during the conduct of the study.** Because hypothesis-testing studies usually are prospectively planned, an overview of the experiments (i.e. the study design) must be included. [Materials and Methods*: A Recipe for Success, MaryAnn Foote, PhD, Chess 2008].

In preparation for the next crash course where you will learn how to write the Material and Methods section: read the below recipe and imagine yourself having to replicate it (like an experiment!). Follow the below guidelines and suggestions to decide whether the recipe has been correctly written.

Homework assignment

1-

Materials and Methods share some similarities with recipes, they both detail steps that you can follow to replicate a dish (or an experiment).

Suggestions: Materials and Methods are usually written in past tense and passive voice. It is usually better to avoid making the Materials and Methods section too long by avoiding unnecessary details.

Here below you can also find the Materials and Methods guidelines from JEI's website:

Materials and Methods

The authors should describe the methods in enough detail such that a different scientist could perform the same experiments and obtain the same results. Materials should not be listed out but should be mentioned within the context of the respective experiment that the materials were used. For example, when explaining a method within this section, the author could state the materials used: “bacteria were grown in standard LB media (FisherSci) for 24 hours at 37°C while shaking.”

By using the above guidelines and the suggestions, try to identify mistakes in the following recipe:

Think about these questions as you read the recipe:

- Are all the important details listed?
- Are unnecessary details present?
- Is the use of verb declination correct?

RECIPE

Homemade burgers:

Ingredients:

500 g minced beef

spring onions

2 cloves of garlic

1 tablespoon of paprika

1 tablespoon of dried parsley

Black pepper (as much as you like)

Buns

Procedure:

I started to prepare the ingredients while listening to some relaxing music. The spring onions have been washed thoroughly and chopped into small pieces with scissors. Discard the roots. All the listed ingredients have been pulled into a bowl, I decided to use a wooden one since I really like them, and the ingredients have been mixed by hand. I preferred to wash my hands at this point, and I used a nice lavender soap to clean them. Once the ingredients have been well mixed, the mixture has been split into 4 equal pieces and each piece has been rolled into a ball and subsequently squashed to obtain a hamburger shape. The burgers have then been placed into the oven.

While the burgers were cooking, I prepared the table by using a tablecloth, prepared the dishes, something to drink and glasses. I have then taken the hamburgers out of the oven and placed them between burger buns.

2-

Get to know your materials!

Here below you can find both a picture of the materials included in your kit with the associated name for each item and a brief description of the main materials included in your kit. Knowing why and how to use them is the key for a successful experiment! When working with new materials, scientists usually read the SAFETY DATA SHEET associated with their material. It provides both information about the purpose/ principles and explanation about the material, as well as storage, instructions and safety information. By using the safety data or the manufacturer's website (follow the links), answer the questions below and get familiar with the material you are using!

Don't forget about the "group glossary"! This glossary will be helpful when you are writing your manuscripts. If you find a word that has not yet been defined or you think your classmates would find it helpful to learn, add it to the glossary.

Tryptic soy broth:

Safety data sheet link: <https://www.bd.com/resource.aspx?IDX=30505>

What is Tryptic soy broth generally used for?

What are fastidious bacteria?

Which are the main components of Tryptic soy broth?

What is sodium chloride important for bacteria?

Agar:

Safety data sheet link: <https://www.sigmaaldrich.com/US/en/product/sial/05040>

Useful wikipedia link: https://en.wikipedia.org/wiki/Agar_plate

Why is agar added to the LB broth (or Tryptic soy broth in our case)?

When talking about agar plates, what does restrictive or selective mean?

Ultrapure distilled water:

Manufacturer website: <https://www.elgalabwater.com/ultrapure-water>

What does ultrapure water mean?

Why is it important to use ultrapure distilled water?

One last tip:

Always wear gloves! Did you know that in a laboratory-setting people are the greatest contributor and account for 46 percent of all particle contamination. Skin flakes, oils, perspiration, and hair can all contribute to cleanroom contamination. And even the most carefully manicured person generates a shroud of particles every day.

Wearing latex or nitrile gloves serves two purposes: to protect you from what you're working with, and to protect what you're working with from you.

Finally, always wear protective equipment, that includes safety goggles. They will protect you from any spill!



Appendix 4: Week 4 activities

Before class: Complete homework assignment from week 3.

In class discussion:

Short lecture to address the following:

- What is a “materials and methods” section and where is it found in a research paper?
- What is the purpose of the materials and methods section?
- What is the “right” amount of detail to include? How is a methods section similar and different from a recipe?
- How can we use a methods section to help our own research?

In small groups facilitators will lead the following activity to help students in drafting their materials and methods section:

- Review exercises 1, 2, and 3 from HW assigned from Week 3
- Part 1: Have the students compare their HW on the recipe. What key information was missing and why? What information was not necessary and why?
- Part 2: Discuss materials, surfaces to be swabbed, review how students will plan to grow their bacteria, etc. in preparation for students to start experiments at home
- Part 3: Review the answers students put for the materials they will be using: what the material is important for, how they will use these safely, etc.
 - Discuss each of the materials in their kits and how they should be used
 - Divvy up experiments between students if necessary

Post-class homework:

- Complete the worksheet below

Introduction to Materials and Methods section continued:

For the next session:

You have started your own experiments, congratulations!

1. In your notebook, try to list all the materials you have used or are going to use for your experimental design. Think about these questions as you draft your list:
 - What material will I use? (examples: Swabs? Sterile water? Number of plates? Broth?)
 - How much of the material did I use? (examples: how many milliliters of the agar stock did I use? how many swabs and plates?)
 - What surface will I test?
 - How will I grow bacteria?
2. Now, write out in bulleted list the steps you used to perform your experiments

You can also use the manuscript used during the first Homework assignment as a guide for drafting your list.

Gehad, Y., & Springel, M. (2020). *Characterization of antibacterial properties of common spices*. Journal of Emerging Investigators. <https://emerginginvestigators.org/articles/characterization-of-antibacterial-properties-of-common-spices/pdf>

Appendix 5: Week 5 activities

Before class: Complete homework assignment from week 4.

In class discussion:

Career panel with invited scientists to discuss their path into science. Questions that students submitted include the following:

- What made you decide you wanted to do X? When did you decide? Did you have a realization moment or was it a gradual realization?
- How did you find the research project/opportunity/mentor which led to your JEI publication?
- How did the JEI writing/publication/peer review process help you prepare for college/career plans?
- How has learning how to read and write scientific literature been helpful for your career?
- How did you look for the right resources, mentors, opportunities to get to where you are today?
- What classes did you take in high school to prepare for college?
- What were most important for your college applications?
- What were some of the biggest challenges you've faced in your path so far? How did you overcome them?
- What are some of your next steps?
- Any last helpful tips or suggestions?

Following the career panel, facilitators will review homework from week 4 with participants.

Post-class homework:

- Students should complete their data collection for their experiment and record quantitative and qualitative data in a table.

Appendix 6: Week 6 activities

Before class: Complete homework assignment from week 5.

In class discussion:

Short lecture addressing the following points:

- What is the results section? Where is it found in a research paper?
- How is the results section organized? Inspect examples such as the one in the image below:
 - This paper is used in appendices 6-8: Carroll K, Coleman T, Yousuf Y, Kim C, Kelberman M. 2022. *Antimicrobial properties of common household spices on microbes cultured from two kitchen locations*. Journal of Emerging Investigators 5:5.
<https://emerginginvestigators.org/articles/antimicrobial-properties-of-common-household-spices-on-microbes-cultured-from-two-kitchen-locations/pdf>

Results Section Example

Our objective was to determine the effectiveness of spices as alternative household cleaners. To achieve this, we quantified microbial growth on agar plates infused with spices that were then inoculated with a subcultured microbe from the refrigerator handle and cutting board. We successfully isolated microbes from our chosen swabbing locations, as demonstrated by visible growth on positive control plates (Figure 1). However, we observed some differences in the amount of area covered by microbes when comparing the three positive control plates for both the refrigerator handle and cutting board (Figure 2). This variation precluded comparisons between different spices, and we ultimately separated analysis into three pairwise comparisons with their respective control plates. There was also evidence of microbial growth over time on positive control plates, as the percent area occupied by microbes increased over the four-day monitoring period (Figure 2). Interestingly, we observed less microbial growth across all positive control refrigerator handle plates compared to positive control cutting board plates (Figure 2). For example, the cutting board control 2 showed approximately 53% coverage on day 1 compared to the refrigerator handle control 2, which showed approximately 38% coverage on day 1. We did not observe any microbial growth on the negative control plates (data not shown).

We observed mixed efficacy of the tested spices to prevent and/or slow microbial growth from swabs of the cutting board and fridge handle (Figure 1). In order to compare microbial growth from control plates with those that contained the spices, we quantified the percent area of the plate covered by microbes from the cutting board and refrigerator handle swabs every other day over the course of a week. Garlic and clove were able to completely prevent the growth of microbes over the course of four days for samples from the refrigerator handle and cutting board (Figure 2). Interestingly, nutmeg completely inhibited the growth of microbes from the cutting board but not the refrigerator handle (Figure 2). However, it appeared as though nutmeg was only able to reduce the growth rate of microbes from the refrigerator handle over the course of four days compared to control plate 1 (Figure 2A). Cinnamon and turmeric both appeared to reduce the rate of microbial growth over the four-day observation period compared to the control plates from the refrigerator handle and cutting board cultures (Figure 2). We were unable to quantify microbial growth on plates containing astragalus due to the rough texture apparent on the plates. However, we did not observe growth of microbes on these plates.

References to the figures

RESULTS

Reminder of scientific question

Brief description methods

Contrary to MM section, here the first Person is used

Report all the observations you made

- Why are figures needed? What do they provide the reader with?
- What is the right figure for your data? Discover examples in the image below

How to choose the right figure for your data

COMPARISONS



DISTRIBUTION



PARTS OF A WHOLE



TREND OVER TIME



DEVIATIONS



RELATIONSHIPS



A bar chart shows data that can be sorted into categories. Each rectangular bar has a height proportional to the value of each category.

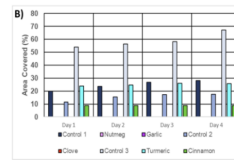
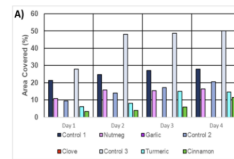


Figure 2: Microbial growth from refrigerator handle cultures. Microbes were cultured from samples obtained from the refrigerator handle (A) or cutting board (B), isolated and subcultured, and streaked onto plates with and without spices. Control replicate one was run alongside nutmeg and garlic plates, control replicate two was run alongside clove and astragalus (data not shown), and control replicate three was run alongside turmeric and cinnamon. Microbial growth was monitored over the course of a week and subsequently quantified as a percent of the total plate occupied.

- Discuss when you should use a table instead of a figure.
- What information should we include in the text of a results section?

Following the lecture, students, with their facilitators will:

- Apply what the students learned from the lecture to their own experimental results.
 - Have the students share results, create data tables from their results, and decide on figures that clearly show the results of their experiments. Facilitators lead discussions on the best figure representation method for their type of data (i.e. bar graphs and pictures of bacterial plates).
 - Refer back to the figures in the JEI article for reference.

Post-class homework:

- Students should write up their results and draft figures of their data.

Appendix 7: Week 7 activities

Before class: Complete homework assignment from week 6.

In class discussion:

Short lecture addressing the following points:

- Why is the introduction of a manuscript important?
- How do I find scientific literature and how do I use it for my own research?
- The following paper is used as an example:
 - Carroll K, Coleman T, Yousuf Y, Kim C, Kelberman M. 2022. *Antimicrobial properties of common household spices on microbes cultured from two kitchen locations*. Journal of Emerging Investigators 5:5. <https://emerginginvestigators.org/articles/antimicrobial-properties-of-common-household-spices-on-microbes-cultured-from-two-kitchen-locations/pdf>

How do I search for scientific literature?

Find some key words that you are interested in in your research

The image shows a Google Scholar search interface with the query 'household bacteria spice'. The search results list several articles, including one by Carroll K et al. (2022) titled 'Antimicrobial properties of common household spices on microbes cultured from two kitchen locations'. A preview of a research article is displayed on the right, titled 'ANTIMICROBIAL STUDY OF NATURAL AND ARTIFICIAL SPICE EXTRACT ON VARIOUS STRAINS OF BACTERIA' by Praga S, Krishna, and Thy K J. The article is published in the International Journal of Scientific & Engineering Research, Volume 10, Issue 10, October 2019, pages 225-232. The article received 121 citations, 2 reviews, and 104 acceptances. The preview also includes the authors' names, affiliations (Department of Microbiology, St. Xavier's College, Kattimunda, Kottayam, Kerala, India), and the journal's ISSN (Print: 1812-1037, Online: 2312-7791).

Start by reading 2-3 related articles, what are they saying about the field in the introduction? What are their conclusions? This gives you an idea about how your research can fill existing knowledge gaps.

- What is the structure of an introduction?

Introduction section example

INTRODUCTION

Bacterial infections are rising, which places a significant burden on the economy and healthcare sector (1). More specifically, foodborne illnesses affect a large portion of the population annually, resulting in an estimated 70,000 hospitalizations and 1,600 deaths in 2011 (2, 3). Of the total number of foodborne illnesses, approximately 39% were a result of bacteria (2, 4). Evidence also suggests that kitchens contain some of the highest amounts of bacteria in a household (5). To minimize the risk of foodborne illnesses, proper sanitization of kitchen surfaces is essential. However, common household cleaners can be expensive and toxic, which has led consumers to seek alternative, ecofriendly, and low-cost cleaning products (6–8).

One promising avenue to pursue as an alternative cleaning solution is the use of spices. Studies have previously demonstrated antioxidant and anti-inflammatory effects of

spices in cells, rodents, and humans, in addition to lowering the risk of some diseases (9). However, evidence also suggests that their use could be expanded past medicinal properties, such as use as antimicrobials. One report tested whether 11 spices could inhibit the growth of *Escherichia coli* (*E. coli*) bacteria (10). 5 of the 11 spices were effective in inhibiting bacterial growth: clove, cinnamon, garlic, oregano, sage, and thyme (10). These results support the antibacterial properties of some spices against specific bacteria. In addition, household spices are natural and easily obtainable; thus, they warrant further study on whether they are effective antibacterial agents, especially in household areas such as the kitchen.

In the present study, we chose to assess three previously tested (garlic, clove, and cinnamon) and three new (nutmeg, turmeric, and astragalus) spices for their potential antimicrobial properties. Although garlic, clove, and cinnamon have previously been shown to be effective at inhibiting the growth of *E. coli*, the kitchen contains a diverse population of microbes on which these spices have yet to be tested. We also chose to test new spices, nutmeg, turmeric, and astragalus, which were not tested by the previous researchers, but have been shown to contain some antibacterial properties. For example, curcumin, which is the primary compound in turmeric, can kill both gram-negative and gram-positive bacteria (11). Meanwhile, evidence suggests that nutmeg might selectively target pathogenic bacterial strains of *E. coli* and *Streptococcus* bacteria (12,13). Astragalus has also been shown to have antibacterial properties (14).

We chose to swab and culture microbes from two different locations in the kitchen: a cutting board and refrigerator handle. We reasoned that different areas of the kitchen may contain different populations and amounts of microbes with variable sensitivities to spices. For example, a cutting board is cleaned after almost every use and may contain grooves after repeatedly being used to prepare food. Through this process, more resistant microbes could emerge through selection. Meanwhile, the refrigerator handle typically is a less often cleaned, highly touched surface, which could suggest that microbes here would be less exposed to common household cleaners and may be easier to kill. In addition to microbial resistance, the overall amount of microbes may be influenced by location. The National Sanitation Foundation (NSF) reported that bacteria thrive in damp environments, such as a cutting board when it is left to dry after cleaning (5). Meanwhile, bacteria grow less efficiently on cool, smooth surfaces, such as a fridge handle

Briefly describe your results

(5) We therefore hypothesized that each household spice would be effective at inhibiting microbial growth from cultures of refrigerator handles but that they might be less effective on samples from cutting boards. We found that cinnamon and turmeric reduced microbial growth from both surfaces, while clove and garlic completely prevented growth of microbes from both locations. Meanwhile, nutmeg completely prevented microbial growth from cutting board cultures but only reduced growth from refrigerator handle cultures, thus demonstrating location-dependent anti-microbial effects. Our results warrant further study into the antimicrobial properties of household spices and their utility as household cleaners.

One concluding sentence about the importance of your research

Briefly explain what have you done and why

Introduce your research

- How do I incorporate references?
- What is the discussion section and how is it structured?

Discussion section example

First paragraph:

provide key findings of your research

And refresh the reader's mind

About the aim of your research

DISCUSSION

In this work, we sought to build upon previous literature on the effectiveness of household spices to inhibit microbial antibacterial properties of various household spices (10). We built on these previous studies in a number of ways. First, we chose to culture microbes from a cutting board and refrigerator handle, instead of using *E. coli*, which we expected would isolate microbes with different susceptibilities to spices. We decided to subculture, as we hypothesized that we would be unable to determine whether spices were effective against a subset of microbes present in a community sample. Thus, subculturing provided a more uniform microbial sample to test within each pairwise comparison. We theorized that growth of microbes cultured from the refrigerator handle might be more vulnerable to spices than cultures from the cutting board, given its suboptimal growing conditions and less frequent cleaning which may lend the microbes less resistance. Second, we tested antimicrobial properties of three spices that had previously been tested against *E. coli* (clove, cinnamon, and garlic), and three spices not previously tested (astragalus, nutmeg, and turmeric). We chose these additional spices based on prior evidence of antibacterial or medicinal properties. Finally, we monitored microbial growth

for four days over the course of a week to ascertain the long-term antimicrobial properties of these spices.

In line with previously demonstrated work, clove and garlic were able to completely prevent the growth of microbes from both cultures over the entire monitoring period. This finding suggests both these spices have short-term antimicrobial properties that last over the course of a week. Interestingly, cinnamon did not prevent microbial growth but was able to slow it over the monitoring period when compared to our positive control (Figure 1). Unfortunately, we were unable to quantitatively describe the effects of astragalus on microbial growth. When pouring the plates, it appeared as though the hot liquid agar reacted with the astragalus and led to a rough texture that made quantification impossible. However, there was a lack of visual evidence of microbial growth throughout the monitoring period. Interestingly, nutmeg completely prevented the growth of microbes from the cutting board culture but only slowed growth of microbes from the refrigerator handle. We also observed what appeared to be 'bubble' growths on both of the turmeric plates, which was reported in a previous study when testing basil (10). These were hypothesized to be fungal in nature, rather than bacterial, and hence brought about our caution in labeling our results as bacteria-specific. In line with this observation, the NSF reported that there was yeast and mold on the refrigerator handle in 23% of households, while there was yeast and mold on the cutting board in 14% of households (5). Curcumin, a compound in turmeric, has antifungal properties and thus supports the notion that these colonies may have been fungal rather than bacterial, given the lack of growth over the monitoring period (15). Overall, our results support selective vulnerability of cutting board cultured microbes only in response to nutmeg. Although the refrigerator handle plates with cinnamon and turmeric showed less growth than the cutting board plates, the positive control plate also showed less growth. Thus, we cannot rule out the possibility that there were different amounts of starting microbes from each subculture. Finally, due to limitations in resources, we were unable to run duplicates or triplicates. These replicates would be necessary to know whether any reduction in microbial growth met statistical significance, though we note that we saw 10-40% reductions in the amount of microbial growth over the observation period.

Following the lecture, students, with their facilitators will:

- Apply what they have learned from the lecture and delineate some keywords for researching primary scientific literature relevant for the introduction section.
- Draft bullet points for the discussion section.

- Conclusions and significance: Discuss the questions they have answered through their experiments and the significance of their findings
- Limitations: Discuss what was not answered through their experiments. Identify the remaining questions and the limitations of their research.

Post-class homework:

- Students should research relevant primary scientific literature for writing their Introduction sections using the key words they identified during class and the ongoing group glossary started on week 2 (Appendix 2).
- Draft the results section and outline the discussion section

Appendix 8: Week 8 activities

Before class: Complete homework assignment from week 7.

In class discussion:

Short lecture addressing the following points:

- What is an abstract and why is it important?
- The following paper is used as an example:
 - Carroll K, Coleman T, Yousuf Y, Kim C, Kelberman M. 2022. *Antimicrobial properties of common household spices on microbes cultured from two kitchen locations*. Journal of Emerging Investigators 5:5.
<https://emerginginvestigators.org/articles/antimicrobial-properties-of-common-household-spices-on-microbes-cultured-from-two-kitchen-locations/pdf>

Abstract Section Example

**Background or Introduction –
What is currently known?**

**Objectives or Aims –
What is the study
and why did you do
it?**

**Methods –
What did you
do?**

**Results –
What did you find?**

**Conclusions –
What did you conclude?**

SUMMARY

The number of bacterial infections in humans is rising, and a major contributor is foodborne illnesses, which affect a large portion of the population and result in many hospitalizations and deaths. Common household cleaners are an effective strategy to combat foodborne illness, but they are often costly and contain harmful chemicals. Thus, we sought to test the antimicrobial effectiveness of readily available, low cost, and relatively harmless spices (clove, nutmeg, astragalus, cinnamon, turmeric, and garlic) on microbes cultured from refrigerator handles and cutting boards. We hypothesized that cutting boards would have microbes that were more resistant to spices given the properties that make them conducive to microbial growth, whereas the microbes cultured from refrigerator handles would be more prone to the antimicrobial effects of the spices. We found that clove, garlic, and nutmeg were able to completely prevent the growth of microbes from cutting boards. Clove and garlic also prevented microbial growth from refrigerator handle samples, but nutmeg only reduced the growth of these microbes over a week. Similarly, turmeric and cinnamon only reduced the growth of microbes over a week from both culture samples. Our results demonstrate long-lasting, antimicrobial effects of multiple spices that support their use as alternatives to common household cleaners.

No references needed
In this section!

- Learn about the peer-review process (submission, reviewer process, re-submission, acceptance).

Activity: Communicate your research findings with your peers

- Students will write their abstracts with their groups guided by their facilitators. Each group should decide how to present their data. For example, students may share their written abstract or choose to present key points from their abstract or paper.
- Student groups will then be paired with another group to share their research questions, hypothesis, results, and conclusions.

Appendix 9: Questions asked in Pre & Post program survey

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [My research will help to solve a problem in the world]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [My findings are important to the scientific community]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [The research question I worked on was important to me]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I faced challenges in completing my research project]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I was able to overcome challenges posed by my research project.]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [The findings of my research project and paper gave me a sense of personal achievement]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I am able to generate a research question to answer]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I am able to figure out what data/observations to collect for a research project]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I am able to create explanations for the results of my science]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I am able to use scientific literature and reports to guide my research]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I have a strong sense of belonging to the community of scientists]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I have come to think of myself as a scientist]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I feel like I belong in the field of science]

The following statements are about your views in doing scientific research, rate the degree to which you agree or disagree with each statement. [I feel that I have a mentor or role model in science whom I look up to or can ask for help]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [Before writing a paper for JEI I was familiar with the process of writing a primary science research paper]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [Writing about my research helps me understand the science better]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [The writing process was challenging for me]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [The JEI mentor was helpful during my writing process]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [The Mini-PhD Program helped me improve my scientific writing]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [I am confident as a scientific writer]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [Submitting a paper through JEI helped me think more carefully about the scientific process]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [Peer review improves the accuracy of the science in a paper]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [Peer review improves the communication of the science presented]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [Peer review can give scientists different ways to understand their science]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [Publication is important because it helps a scientist share his or her science with a broader audience]

The following statements are about the role of writing, peer-review and publication within science. Rate the degree to which you agree or disagree with each statement [Publication is important because the science in a paper could be used by another scientist in his or her project]