



REVIEW ARTICLE

ROOTING OUT RESISTANCE: INVESTIGATING ANTIBIOTIC RESISTANCE IN PLANTS

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ABSTRACT

For decades, livestock have been fed antibiotics to promote growth, treat infections, and prevent diseases in crowded and often unsanitary conditions. "Worldwide it is estimated that 66% of all antibiotics are used in farm animals, not people." (ASA 1). However, the extensive use of these antibiotics has resulted in the emergence of highly drug-resistant bacterial pathogens. Antibiotic Resistance is caused when bacteria change to resist the antibiotics used to treat them effectively. If antibiotics are not taken promptly or if doses are skipped, then the bacteria grow to develop resistance. This led us to our research question: Can antibiotic use in livestock affect antibiotic resistance found in radishes if they are grown in organic manure-based fertilizer? Through the application of previous studies and research, we conducted our experiment to analyze antibiotic resistance in plants grown in animal-based manure.

INTRODUCTION

Antibiotic resistance is a critical public health issue characterized by the ability of bacteria to withstand the effects of antibiotics that once killed or inhibited their growth. This phenomenon was first identified by Alexander Fleming in 1928 when he discovered penicillin, the first antibiotic. Over the subsequent decades, the widespread use and misuse of antibiotics in medicine and agriculture accelerated the development of resistant bacterial strains. Antibiotic resistance occurs when bacteria evolve to survive exposure to antibiotics designed to kill them or inhibit their growth. This resistance is primarily caused by the overuse and misuse of antibiotics in both human medicine and agriculture, which applies selective pressure on bacteria, encouraging the survival of resistant strains. Additionally, bacteria can acquire resistance through genetic mutations or by obtaining resistance genes from other bacteria via horizontal gene transfer. These factors combined lead to the proliferation of antibiotic-resistant bacteria, posing a significant challenge to public health. Today, antibiotic resistance poses a significant threat to global health, as it leads to longer hospital stays, higher medical costs, and increased mortality. The ability of bacteria to evolve and render antibiotics ineffective endangers the efficacy of treatments for common infectious diseases, making it imperative to address this issue through global cooperation, stringent antibiotic stewardship, and the development of new antimicrobial agents.

Hypothesis: Can antibiotic use in livestock affect antibiotic resistance found in radishes if they are grown in organic manure-based fertilizer?

METHODS

Materials

- Vegan Fertilizer
- Organic Fertilizer
- Organic Cow Manure
- Radish Seeds
- Three 9 inch(depth) pots
- Regular planting soil
- 10 agar plates
- 12 nutrient agar bottles (3.2 mL per plate)
- Gloves
- Pipettes
- Stir rods
- E. coli (gram negative)
- Bacillus cereus (gram positive)
- Streptomycin Antibiotic Disc
- Neomycin Antibiotic Disc
- Radish seedlings grown in organic fertilizer
- Radish seedlings grown in vegan fertilizer
- Radish seedlings grown with no fertilizer

Procedure

Description of sampling methods: 6 seeds were planted in each pot labeled vegan (with vegan fertilizer), control (with no fertilizer), and animal (with cow manure-based organic fertilizer).



The pots were placed into a plant incubator where they were initially watered twice for two weeks. After the saplings emerged and started to stand firmly on their own, they were watered once a day.



- Once the saplings had grown, they were prepared to be tested with bacteria.
- Next, we began our testing procedures. For Trial 1, the 10 plates were divided into equal sections in which the experiments were placed into the agar plate. Five plates were used to test the samples in the bacteria *E. coli* and five of the plates were used to test samples in the bacteria *Bacillus Megaterrium*. Then, the plates were placed in an incubator for 48 hours - due to the amount of the sample - at 30 degrees Celsius.
- For Trial 2, We utilized filter paper - a method widely used for enumerating substances in samples - to place the samples of the bacteria, soil, and saplings in the agar plates. Once the agar plates were set, we used a hole punch to cut out the filter paper into sample sizes.
- The filter paper samples were covered/dipped into either the solid experimental (soil) or a slurry of the plant sample respectively/ bacteria.
- Next, we marked the agar plates and placed the samples even across the agar plates. Five agar plates were used to test the samples in *E. coli* and the other five plates were used to test the samples in *Bacillus cereus*. Alongside the samples, discs of antibiotics were also placed as a control. We then placed the plates in an incubator for 24 hours at 30 degrees Celsius.

Data collection/ analysis: Due to the nature of this experiment, quantitative data was not applicable. Therefore, observations were noted.

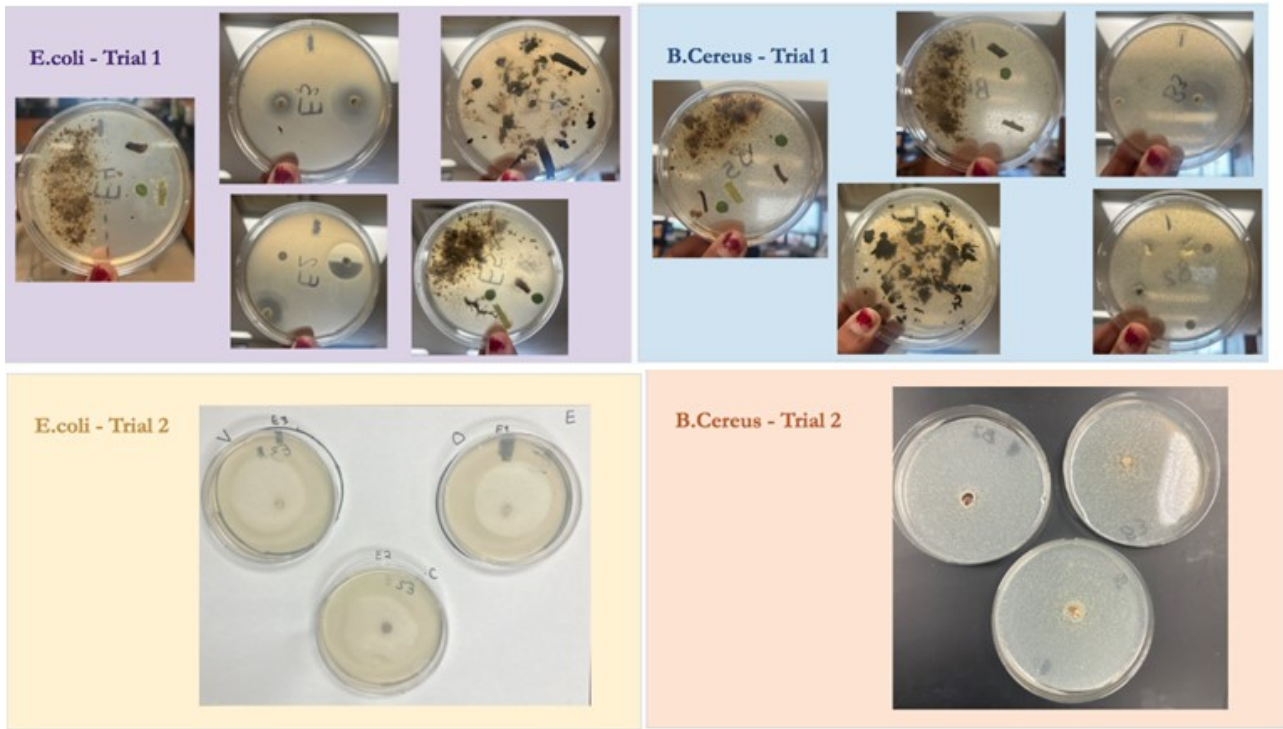
RESULTS

The observations of our two trials indicate that the radishes fertilized with organic manure from antibiotic-treated livestock nor the soil/ fertilizer which is suspected to carry antibiotic properties do not show a higher prevalence of antibiotics than radishes fertilized with vegan fertilizers due to the absence of a zone of inhibition.

DISCUSSION

Interpretation: A zone of inhibition is a clear area surrounding an antimicrobial agent on an agar plate that indicates the extent of antimicrobial activity against a test microorganism. It is used to assess the efficacy of antibiotics and other antimicrobial substances. The absence of antibiotic resistance against *E. coli* and *Bacillus cereus* bacteria isolated from the radish plants/ fertilizer samples suggests that these plants are not contributing to the propagation of antibiotic-resistant strains. Gram-positive bacteria (*E. coli*) have a thick peptidoglycan layer in their cell walls and no outer membrane, appearing purple after Gram staining while Gram-negative bacteria (*B. cereus*) have a thin peptidoglycan layer and an outer membrane, appearing pink/red after staining. Testing antibiotic resistance in both types was crucial because their structural differences affect how antibiotics penetrate and act on them, leading to varied resistance mechanisms and influencing effective treatment strategies for different infections. This finding is significant as it indicates that the use of antibiotics in the environment where these radishes were grown has not led to the development of resistance in these bacterial species. It also implies that radishes, in this particular context, could be considered safe from the perspective of transferring antibiotic-resistant bacteria to humans or other plants. This information can help guide agricultural practices and inform strategies to prevent the spread of antibiotic resistance in both the environment and the food chain. Findings on antibiotic resistance often reveal a troubling trend of increasing resistance among various bacterial species, particularly those commonly associated with human infections such as *E. coli* and *Staphylococcus aureus*. These findings highlight the widespread impact of antibiotic misuse in healthcare and agriculture, leading to resistant strains that pose significant treatment challenges. In contrast, the interpretation of the results showing no antibiotic resistance in *E. coli* and *Bacillus cereus* isolated from radish plants offers a glimmer of hope. It suggests that not all environments or agricultural practices contribute equally to the problem of antibiotic resistance. This comparison underscores the importance of identifying and promoting agricultural practices that do not exacerbate antibiotic resistance, as well as the need for continuous monitoring to understand the varying impacts of different environments and practices on the development of resistant bacterial strains.

Limitations: An experiment testing antibiotic resistance in radish plants has several limitations and weaknesses. Firstly, the scope might be limited to specific bacterial strains such as *E. coli* and *Bacillus cereus*, not accounting for other potentially resistant bacteria. Additionally, environmental factors, such as soil composition and agricultural practices, may vary and influence the results, making it difficult to generalize findings.



The sample size and geographic location of the plants tested could also limit the applicability of the results. Secondly, the experimental conditions may not accurately reflect real-world scenarios, potentially impacting the validity of the conclusions.

Future Research: We hope to continue this project by testing in the future under different conditions through the utilization of different variables and to do more data analysis using quantitative data.

CONCLUSION

In summary, from the observations of our two trials, we have concluded that the radishes fertilized with organic manure from antibiotic-treated livestock do not show a higher prevalence of antibiotics than radishes fertilized with vegan fertilizers.

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