# Meropenem Resistant Bacteria in Chicken Coop Soil

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## Abstract

Antibiotic resistance in bacteria has become an increasing concern in healthcare settings. Our study investigated chicken coop soil samples for presence of bacteria resistant to meropenem, a last resort antibiotic used in hospitals. We used MAC agar plate cultures to grow bacteria in the presence of meropenem. In this experiment, found the presence of antibiotic-resistant bacteria (ARBs) in all four samples tested.

## Introduction

Carbapenems are a class of antibiotics used only in hospital settings as a last resort to treat infections that are not responding to other antibiotics. Resistance to these antibiotics poses serious public health concerns which limit infection treatment options and increase mortality rates (Meletis, 2016). Farming practices have been identified as a major source of resistance due to frequent use of antibiotics, as well as the geographical spread of products. A recent study has found evidence of resistance to carbapenems within both agriculture and urban poultry farms (Lopez, et al., 2020).

The purpose of this project was to further research resistance to carbapenems in poultry farms by testing agricultural and urban farms samples in the presence of meropenem. We sampled four locations (two agricultural, two urban) and cultured samples. We then used PCR and AGE techniques to isolate and sequence the 16s rRNA gene to identify the species.

## Methods

### *Materials & Location*

Materials: MAC agar plates w/ nystatin x24 (12 mero, 12 N/A), micropipettes + tips, parafilm, analytical balance, PCR (DH20, 320F & 817R primers, tubes), Agarose Gel Electrophoresis kit (1x TAE buffer, gel dye). Sample locations:

A- (48.34447125907454, -122.39247943148537)

B- (48.859662219956306, -122.45398637901948)

C- (48.754402125005875, -122.4154691356018)

D- (48.763463613961044, -122.47452483702178)

### *Procedure*

We began by sampling four locations for soil. Then, we measured 1g of soil and created dilutions of 1/10, $1/10^2$, $1/10^3$, $1/10^4$ and $1/10^5$. For each sample location, dilutions were plated as follows: 1/10, $1/10^2$, and $1/10^3$ on MAC agar mero. $1/10^3$, $1/10^4$ and $1/10^5$ on MAC agar N/A. After culturing we sampled 2 from each location for PCR and AGE. Upon successful, PCR samples were sent for BLAST sequencing and identification.

## Bacterial Colonies by Location

| Location | Count | Dilution |
|---|---|---|
| A<br>Mt Vernon, WA | 52 | $1/10^3$ |
| B<br>Lynden, WA | 52 | $1/10^2$ |
| C<br>Bellingham, WA | 10 | $1/10^2$ |
| D<br>Bellingham, WA | 94 | $1/10^3$ |

*Table 1*. Results of MAC agar bacteria cultures in the presence of meropenem.

## Meropenem Resistant Bacterial Growth

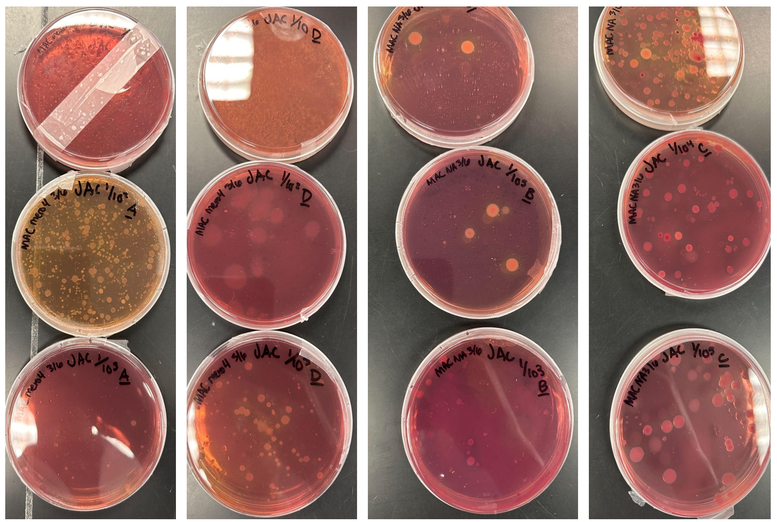

*Figure 1. Bacterial growth on MAC agar plates in the presence of meropenem at 3 dilutions.*

## Discussion

For each soil sample (A-D), colonies had formed on the meropenem resistant plates (fig 1.). For samples A ($10^3$), B ($10^2$) and D ($10^3$), the plates showed an abundance of bacterial growth, with sample A and B showing 52 colonies and sample D showing 94 colonies. In contrast, Sample C ($10^2$) had fewer colonies with around 10 visible (table 1.).

The presence of growth on the plates treated with meropenem indicates that these samples contain antibiotic-resistant bacteria, which we aimed to further analyze by pinpointing specific genes. *P. aeruginosa*, a bacteria species known for its resistance to nearly all antibiotics was identified in all DNA samples (Meletis, 2016). By comparing the use of fertilizer in agriculture vs. urban environments, it gives us an insight of how different environments use and contribute to the presence of antibiotic resistance. The growth of ARBs in commercial farm soils suggests that these operations contribute to the spread of antibiotic resistance due to the routine use of antibiotics in livestock (Lopez, et al., 2020). In contrast, private farm soils exhibit lower levels of bacterial growth in the presence of meropenem, which could indicate reduced antibiotic use or difference in soil composition. this also suggests urban locations also are likely influenced by wastewater, human activity, or environment contamination which led to antibiotic resistant bacteria.

There were some limitations of this study, including number of sample locations and antibiotics tested. For future studies, we should consider a wider range of antibiotics and variety of farm environments to provide a more in depth understanding of antibiotic resistance in fertilizer. Additionally, further investigation into the mechanisms by which bacteria are resistant could help us understand prevention and treatment.

Our findings support the hypothesis that bacteria resistant to meropenem are present in both urban and agricultural chicken coop soils, indicating how antibiotic resistant is used in all environments (whether urban or agriculture).

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## References/ Work Cited

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