Agricultural Soils in Whatcom County Are a Source of Carbapenem Resistant Bacteria

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Abstract

Carbapenems are a class of last resort antibiotics that are used across the board in healthcare facilities as a means of treating multidrug-resistant infections. To date, the majority of studies dealing with Carbapenem-resistant bacteria (CRB) have had a focus on medical locales. Because of this there is relatively little published research in the scientific literature about the prevalence of CRB's in the environment. In our study we set out to survey 3 different agricultural sites in Whatcom County to ascertain if these antibiotic-resistant bacteria were present in the environment.

Introduction

The most used antibiotics in the world are Beta-lactams. Amongst these types, Carbapenems' are the most effective against Gram-positive and Gram-negative bacteria. Thus, Carbapenems are considered a last resort bacterial treatment (Meletis 2016). The Centers for Disease Control and Prevention (CDC) has assigned Carbapenem Resistant Bacteria (CRB) as a major public threat due to its difficulty in treating the infections .However, carbapenem-resistant bacteria (CRB) in the environment in the United States is relatively unknown. A study on the Prevalence of CRB in California was found mainly in agricultural locations (Lopez Et al 2020). No studies were found in Washington state. To contribute to this information gap, this study aims to find the prevalence of CRB in agriculture areas in Whatcom County, using a commonly administered Carbapenem, Meropenem, which the WHO lists as an essential medicine (Armstrong Et al 2021). We tested a private and commercial chicken farm, as well as a cow farm due to there being findings of CRB in southern California at these locations. (Lopez, N. V. et al.) Our hypothesis was that in these settings there would be a higher probability of antibiotic-resistant bacteria based on the understanding that antibiotic use is common among livestock farms, especially those where high populations and close quarters may lead to increased disease transmission. In particular, Enterobacteriaceae, *Pseudomonas aeruginosa,* and *Acinetobacter baumanii,* due to these bacteria's being identified by the CDC as CRBs' (Lopez Et al 2020).

Methods

Materials & Location

Materials: 3 different soil samples, 3 plates each for the following dilutions on MacConkey + Amphotericin + Meropenem; 1/10^1,1/10^2, 1/10^3, 3 plates each for the following dilutions on MacConkey + Amphotericin; 1/10^4, 1/10^5,1/10^6, 30°C incubator, 20 µl prewarmed Hotshot solution, Sterile microfuge tube containing NaOH, detergent, and calcium chelator, Sterile pipette tips, Microfuge tube containing heated 5X HS LB, Vortex, Tabletop centrifuge, 20 µl of 5X neutralization solution, Nuclease-free water, Gel box, Agarose gel, Parafilm, Loading buffer, 1 kb DNA ladder, 1X TBE buffer, Ethidium bromide (EtBr) staining solution, UV light box, Tube with 4 µl of Exo-SAP-IT, ice, thermocycler, device to do blast search.
Location: Whatcom Community College, Kulshan 101, Soil sample coordinates; 48.83410 N, 122.51543 W (Cow Farm), 48.78793 N, 122.57721 W (Private Chicken Farm), 48.85975 N, 122.45390 W (Commercial Chicken Farm)

Procedure

The following procedure was adapted From Lab 4- PARE PROJECT and Lab 5 –Identification of Bacterial Unknown by 16S rRNA gene sequencing;

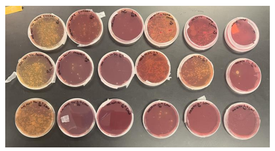

Figure 1. Plated serial dilutions for each soil sample, 1/10^1-1/10^6

1.Perform serial dilution for each soil sample
2.Plate the samples and incubate at 30 degrees Celsius for 72 hours
3.Select 2 plates with 30-300 colonies from each sample for counting
4.Extract DNA from selected bacterial colonies using HotShot solution.
5.Set up PCR to amplify the 16S rRNA gene, following standard thermocycling protocols.
6.Analyze PCR products on agarose gel stained with EtBr and visualize DNA bands under UV light.
7.Calculate CFUs per gram of soil
8.Analyze DNA sequences using NCBI BLAST to identify bacterial strains.

Sample Locations and Colony Forming Unit per Gram

Location Type	Location Coordinates	Total cfu/g (# of viable microorganisms in a sample)	% CRB
Cow Farm	48.83410 N, 122.51543 W	8.5E5	1.059%
Chicken Farm	48.85975 N, 122.45390 W	6.5E8	0.00761%
Private Chicken Farm	48.85975 N, 122.45390 W	4.5E9	0.0308%

Table 1. This table presents microbial data collected from three agricultural locations, focusing on the presence of **carbapenem-resistant bacteria (CRB)**. The sites include a **Cow Farm**, a **Commercial Chicken Farm**, and a **Private Chicken Farm**, all located within a close geographic range.

Figure 2: This map shows the three locations in Whatcom County we collected our soil from

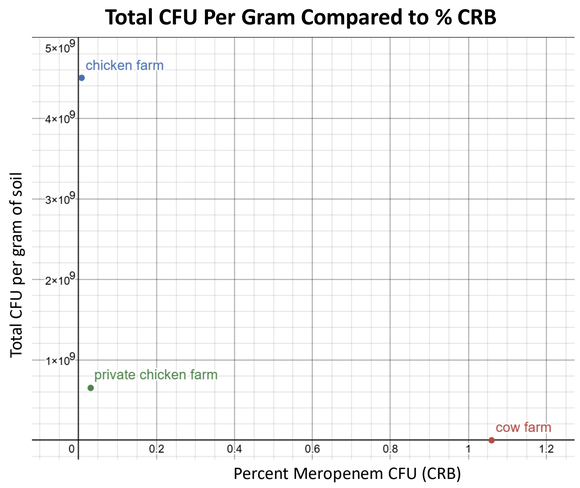

Figure 3. The x-axis represents the percent of Meropenem CFU, and the y-axis represents total cfu per gram of soil. All three samples are shown in this figure.

Discussion

Of the three locations we took soil samples from, all three came back positive for Carbapenem resistance. The first sample we tested was from a commercial cow farm, and the soil was taken from inside the pasture. (48.83410 N, 122.51543 W). This came back with 8.5x10^5 total CFU/g and 1.059% Carbapenem resistant bacteria, further testing of two colonies from this plate was inconclusive on specific bacteria. The next sample we tested was from a commercial chicken farm, this soil was collected right next to the chicken pasture approximately a foot away (48.85975^0 N, 122.45390^0 W). This came back with 6.5x10^8 total CFU/g and 0.00761% Carbapenem resistant bacteria. One of the two colonies tested on this sample was genetically sequenced and came back as an unidentified species of Pseudomonas bacteria, this bacteria can infect a wide variety of hosts including humans and is extremely difficult to treat as it is antibiotic resistant. Severe infection from this bacteria can lead to respiratory failure, shock and even death (Wilson & Pandey, 2023). The second testing of a bacterial colony on this sample was not found to be in the nucleotide blast meaning that it could have been more distantly related to the other Pseudomonas bacteria and not in the data base or that it perhaps was just not a good sequence. The final sample we tested was from a private family chicken coop, soil was taken from inside the coop (48.85975 N, 122.45390 W). This came back with 4.5x10^9 total CFU/g and 0.0308% Carbapenem resistance, further testing of two colonies was inconclusive. Through this research although we were not able to successfully identify all species present in our samples we did prove our hypothesis that agricultural soils are indeed a reservoir of carbapenem resistant bacteria in the environment. Further testing needs to be done to gather a broader view of CRB's in Whatcom County soils and Washington State as a whole. The implications of this study are concerning and pose a direct public health risk. CRB's are potentially wide spread and most likely present in soils all across our county including urban areas that we weren't able to test given our resource and time constraints. We hope that this research can serve as a blueprint for future studies on this topic locally and nationally and provide medical experts and state organizations with a source of information that they can use to help better regulate the distribution of these antibiotics.

Acknowledgements

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