

Antibiotic Resistance within The Village Agriculture Cooperative, A Community Science Project

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Abstract: Antibiotic development and use have dramatically extended the average human lifespan and revolutionized modern medicine. Antibiotics facilitate prophylactic reduction in surgical site infection and reduce the mortality of chemotherapy patients. However, antibiotic resistance poses a severe hindrance to the continued effectiveness of antibiotic treatments. The presence of a large healthcare industry in Rochester, Minnesota, raises the possibility of increased antibiotic-resistant genes in the environment. Furthermore, historical livestock practices within Rochester's local farms may further exacerbate antibiotic resistance as antibiotics may be used to treat active infections as well as prophylactically. Coupled to this is grower land use practices which include the application of animal waste as fertilizer. This study investigated bacterial abundance, the prevalence of antibiotic resistance, and antibiotic-resistant species found in community farm soil in Rochester, Minnesota; Finding a large population of resistant *Priestia megaterium* across all three sites sampled with the highest prevalence at the oldest agricultural site sampled, the History Center. Elucidation of the current microbial makeup of the garden plot soils paves the way for future efforts to identify mitigation efforts; these efforts include targeting local environmental policy surrounding antibiotic stewardship in Rochester, MN of anthropogenic activities which may contribute to increased microbial resistance.

Introduction

A worldwide public health challenge lies in the spread of antibiotic resistance (9). By 2050, estimates indicate that the rise in antibiotic resistance will increase the death rate due to resistant bacterial infections from 700,000 to 10 million per year (9). In the United States, antibiotics are used to treat food-

producing animals (10). Likewise, antibiotics are applied to agricultural plants to prevent pathogen growth (11, 12). Contact with produce treated with antibiotics can lead to antibiotic-resistant pathogens (13).

Soil is a major reservoir of antibiotic resistance gene determinants as the allelic variants found in pathogenic bacteria can be

found within soil-dwelling bacteria (5, 2). This is in part because most antibiotics used today have been isolated from soil-dwelling bacteria, especially actinomycetes, which comprise most currently used drugs developed in the 1940s to 1960s (3). The antibiotic production of the soil microbiome can lead to diverse production of antibiotic resistance genes in the bacterial population (4). In the soil, these antibiotic-resistance genes are an essential part of the environmental antibiotic resistome (6, 7). An explanation for the high incidence rate of antibiotic resistance genes is the arms-shield hypothesis, which states that various lineages of bacteria can produce similar antibiotic resistance genes to counteract the antibiotics produced in their environment (8, 5).

Surveillance of antibiotic resistance provides a tool for risk assessment, informing the epidemiology of antibiotic resistance, especially as antibiotic resistance continues to be amplified through anthropogenic inputs (1, 5, 4, 6, 8, 9). As such, this project aimed to contribute to antibiotic resistance surveillance within The Village Community Gardens (VCGs) in Rochester, MN. VCGs is a local nonprofit aiming to foster food sovereignty and provide land and resources to cultivate culturally relevant foods (14). At VCGs, individual community gardeners employ various fertilizer practices, and each garden site has a unique history of land use. These factors can lead to the compounding of the agricultural soils' naturally occurring antibiotic resistance genes through the potential inclusion of farming practices such as the application of manure, irrigation, and the use of antibiotics to treat crop disease (15, 6, 16, 17, 12).

We aimed to elucidate the abundance of antibiotic resistance for ampicillin and spectinomycin within the soil of VCGs and investigate the resistant bacterial species present. Ampicillin is commonly used for human and veterinary antibacterial therapies (18, 19). Incomplete metabolism coupled with overuse has contributed to elevated levels in wastewater (20, 21). Ampicillin became commercially available in 1961, and its use is expected to increase (22). Spectinomycin was historically used to treat *Neisseria gonorrhoea* in humans, but as of 2019, the Food and Drug Administration discontinued its use for the treatment of human disease (23, 24). However, the use of spectinomycin remains approved for the treatment of bacterial enteric and respiratory infections in livestock (25). Ampicillin and Spectinomycin resistance were selected for comparison due to the varied anthropogenic use and, thus, antibiotic pollution potential of these two therapies.

Methods

Study Area:

This study was carried out in Rochester, MN, over the course of three months, from September 2024 to November 2024, in cooperation with the local nonprofit Village Community Gardens (VCGs). VCGs has six total gardening spaces. The three oldest and largest garden plots were evaluated: Jubilo Farm, Rochester History Center, and Rochester Covenant Church.

Sampling method:

Simple random sampling was used to collect six ¼ cup surface soil samples within each of the three garden plots. Sampling points

were established using a grid, and sites were apportioned using a random number generator. The soil samples were placed into a sterile collection vehicle. These pooled samples were then mixed via physical agitation, and a representative sample was created. The soil was collected up to one day prior to experimental set-up and stored at 4°C. This was done to ensure that every member of the bacterial population had an equal chance of being selected, minimizing bias and providing an accurate reflection of the larger population.

Enumeration of Resistant Soil Bacteria & Microbial Biomass:

Trypticase soy agar (TSA) plates with 100 µg/mL Ampicillin and 50 µg/mL Spectinomycin were prepared along with control TSA plates. Soil samples from the three collection sites were serially diluted 10-fold in DI water until a dilution of 10⁻⁸ was reached. The impregnated plates were plated with 100 µl of suspended diluted samples, ranging from 10⁻¹ to 10⁻³. Regular TSA plates were plated with the diluted samples ranging from 10⁻⁴ to 10⁻⁸. After incubating the TSA plates, colony-forming units (CFUs) were determined.

Identification of Bacterial Species using 16S rRNA:

To elucidate which species were antibiotic-resistant in the farm plots, 16S rRNA of the bacterial species cultured were amplified through PCR to allow for identification of the species through Sanger Sequencing. Morphologically distinct colonies (bacteria exhibiting different phenotypes based on color, shape, size, texture, and consistency) were picked among antibiotic-impregnated TSA

plates via a sterile pipette tip and mixed in the PCR mastermix. The PCR mastermix consisted of 12.5 µl 2X taq master mix (Promega), 0.5 µl of each primer (0.5 µM); 27f & 1492R, and 11.5 µl water. PCR was then performed with an initial denaturing phase of 5 minutes at 94°C before 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute. Lastly, a final extension was performed at 72°C for 5 minutes.

Gel Electrophoresis & Sequencing:

Gel electrophoresis was performed to evaluate PCR amplification success. Five µl of PCR product was visualized on a 2% agarose gel, stained with SYBR Safe DNA Stain (Edvotek), in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). PCR products were run at 80V for 45 to 50 minutes. Results were visualized using a UV transilluminator. PCR samples that contained a single band at the expected PCR product size of around 1,500 base pairs were selected for Sanger sequencing (Eurofins).

Sequence Analysis:

Chromatograms were analyzed, and the National Institute of Health Nucleotide BLAST was used to identify isolated species. Chromatograms are a visual representation of the DNA sequence results; visual inspection of peak shape, width, and height assured confidence in the correct nucleotide base call. DNA sequences were aligned with the ClustalW multiple sequence alignment method (26), and a phylogenetic distance tree was constructed using the Clustal W alignment method (DNASTAR) comparing 16S rDNA sequences was created.

Statistical analysis:

GraphPad Prism and Excel were used to produce Figures 1 and 2. With the exception of the standard deviations produced by GraphPad Prism in Figure 1.A and Figure 1.B, all statistical analyses were performed using Microsoft Excel. Single-factor ANOVA tests were performed to determine if there were significant differences between the groups. Bonferroni corrections were performed as the post-hoc analysis of the single-factor ANOVA results. Specifically, two-sample t-tests assuming unequal variances were performed, comparing all the groups involved in the ANOVA analysis to determine where the significant differences were.

Results

Soil Bacteria & Microbial Biomass:

Spectinomycin-resistant bacteria were found in the greatest concentration at the agricultural plot of the History Center (Fig. 1.B), while ampicillin-resistant bacteria were in the highest concentration in the soil of Jubilo Farm (Fig 1.A). Rochester Covenant Church had the lowest ampicillin resistance and second highest spectinomycin resistance. For bacterial biomass, the highest concentration was Jubilo Farm, which had close to 1.95E6 CFU/ml, while the lowest was History Center at 0.42E6 CFU/ml (Fig. 1.C).

To account for the difference in total bacterial biomass between the sites, the amount of antibiotic-resistant bacteria was standardized to the total bacterial biomass of their respective agricultural sites to give an idea of what percentage of the bacterial population is antibiotic-resistant (Fig. 2). Based on these percentages, further statistical analysis was conducted. It was found that assuming 95%

confidence ($\alpha = 0.05$), History Center had significantly higher ampicillin antibiotic resistance within its bacterial population than Jubilo Farm ($p = 0.018$) and Rochester Covenant Church ($p = 0.037$). However, there were no significant differences between Jubilo Farm and Rochester Covenant Church ($p = 0.238$). Looking at spectinomycin resistance, again with 95% confidence ($\alpha = 0.05$), History Center had significantly higher spectinomycin-resistant bacteria within its bacterial population than Jubilo Farm ($p = 0.044$). However, no significant differences were found between History Center and Rochester Covenant Church ($p = 0.055$) or Jubilo Farm and Rochester Covenant Church ($p = 0.193$).

Bacterial Species Identified using 16S rRNA

Among the ampicillin and spectinomycin-resistant species, the bacterial species primarily identified was *Priestia magisterium*. Furthermore, among the ampicillin-resistant species, *Pseudomonas* species were identified. Among the controls, a variety of species were identified, as would be expected without the selection of the antibiotics (Fig. 3).

Discussion

In this study, the resistance to two antibiotics, ampicillin and spectinomycin, was investigated. Both antibiotics play key roles in agriculture and medicine, making resistance to them a major public health concern (10). Different agricultural management practices and historical land use impacts bacterial communities and their resistome (15, 6, 16, 12). Raw produce carrying antibiotic-resistant bacteria can expose humans to antimicrobial-

resistant bacteria upon consumption (27). However, antibiotic-resistant contamination of soil and the associated risk to human health has yet to be elucidated.

The highest prevalence of antibiotic resistance for both antibiotics, relative to the other sites, was found at the History Center. Soils within the History Center yielded a higher percentage of ampicillin resistance than the other two sites and had a higher spectinomycin resistance percentage than Jubilo Farm. Furthermore, Jubilo Farm and the History Center had no statistically significant difference in the percentage of bacterial population being antibiotic resistant between them. This result may originate from the considerable amount of time in which the agricultural plot at the History Center has been farmland, having been as such since 1856 (28). Anthropogenic inputs into crop production at this 169-year-old agricultural plot of land, including use of manure, irrigation, and antibiotic application against plant pathogens, may have contributed to this observable elevated antibiotic resistance rates (15, 17, 6, 16, 12).

Among the species identified with Sanger Sequencing, *Prieta megaterium* was identified as the main resistant bacterial species for both antibiotics across all three plots. This may be due to the spore-forming, with Gram-positive bacterium being a species likely to have persister cells that can survive antibiotic stress

for extended periods of time (29). Fortunately, while there are rare cases of infection through *P. megaterium*, it is unlikely to be pathogenic and, in fact, even exhibits antimicrobial activity against some plant pathogens (30, 31).

Overall, this study elucidated bacterial abundance, prevalence of antimicrobial resistance, and identified resistant species within Rochester, MN, community gardens. Elucidation of the current microbial makeup of the garden plot soils paves the way for future efforts to identify mitigation efforts; these efforts include targeting local environmental policy surrounding antibiotic stewardship in Rochester, MN, and anthropogenic activities which may contribute to increased microbial resistance (6, 12, 15, 16). This data can further be employed to inform those cultivating the farms on their antibiotic exposure potentials and make steps towards enhancing awareness on the growing global problem of antibiotic resistance (9). This study supports the growing evidence that community gardens harbor bacteria with antimicrobial resistance mechanisms. Future anthropogenic studies aiming to elucidate the source(s) of antimicrobial resistances would allow for local policy formation to mitigate and remove continued input and formation of genes that confer antimicrobial resistance.

Figures

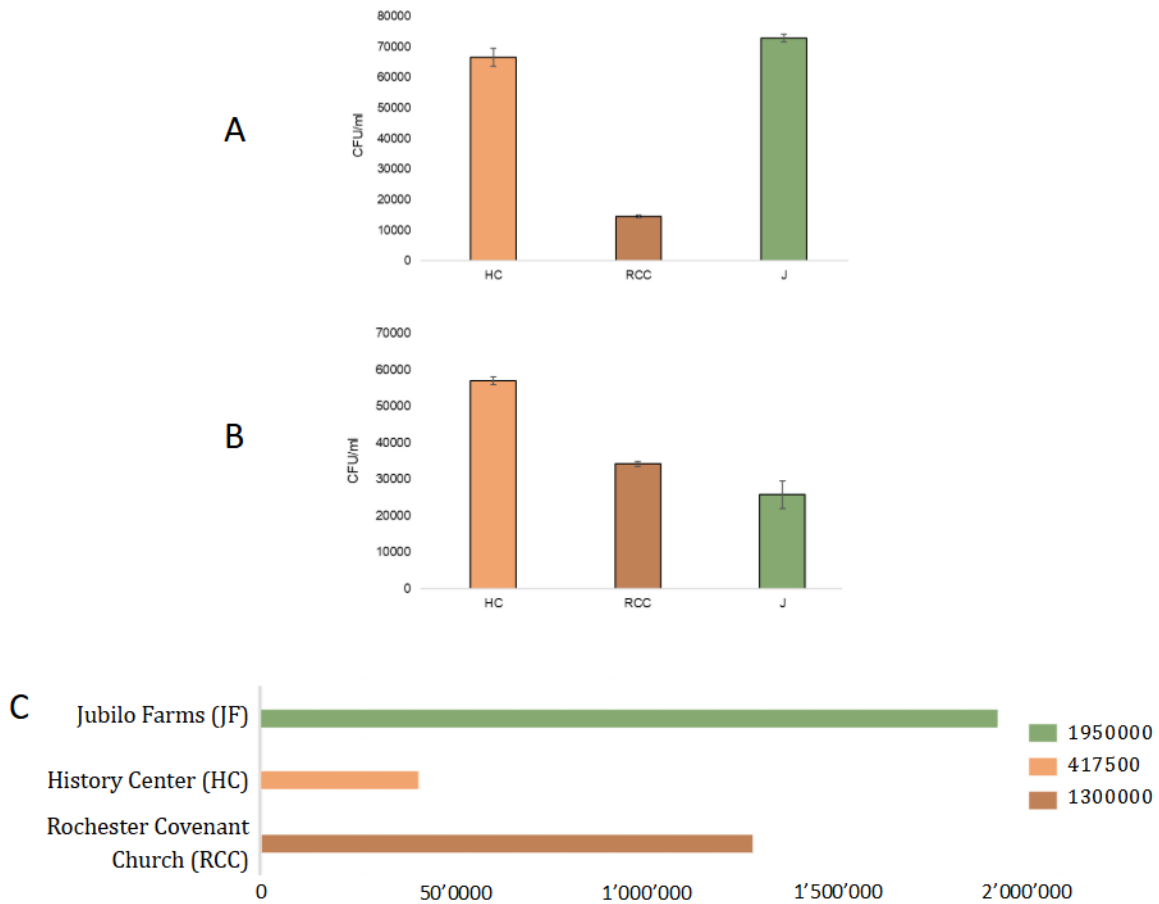


Figure 1. (A) Prevalence of the ampicillin-resistant bacteria at HC (History Center), JF (Jubilo Farm), and RCC (Rochester Covenant Church). The error bars represent SD. (B) Prevalence of spectinomycin-resistant bacteria in the soil at HC, JF, and RCC. The error bars represent SD. (C) Total biomass of bacteria found at each location. A,B, and C are quantified in CFU/ml.

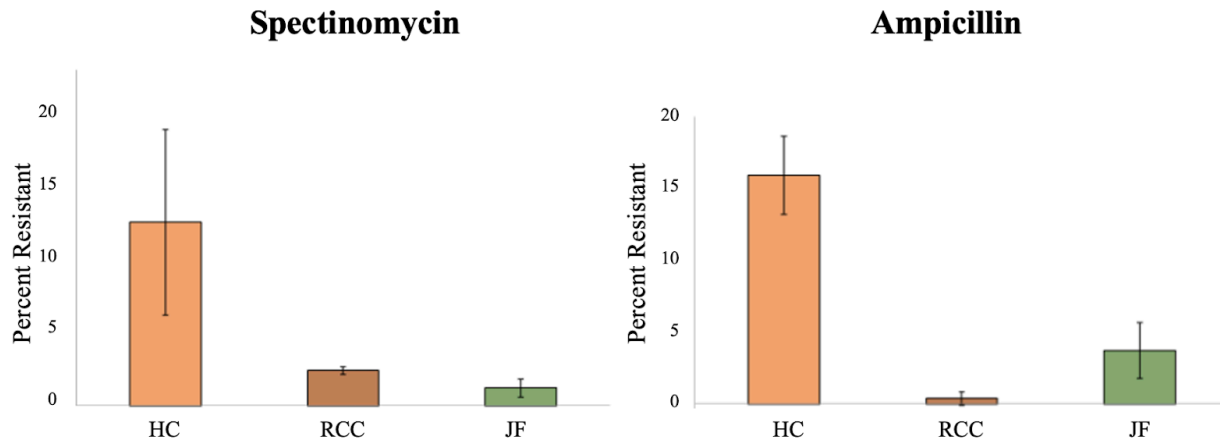


Figure 2. Percentage of the total bacterial population resistant to spectinomycin or ampicillin at each sampling site (HC = History Center, RCC = Rochester Covenant Church, JF = Jubilo Farm). The percentage of bacteria resistant to spectinomycin is depicted on the left, while the percentage of ampicillin-resistant bacterium is depicted on the right. Errors bars represent standard deviation.

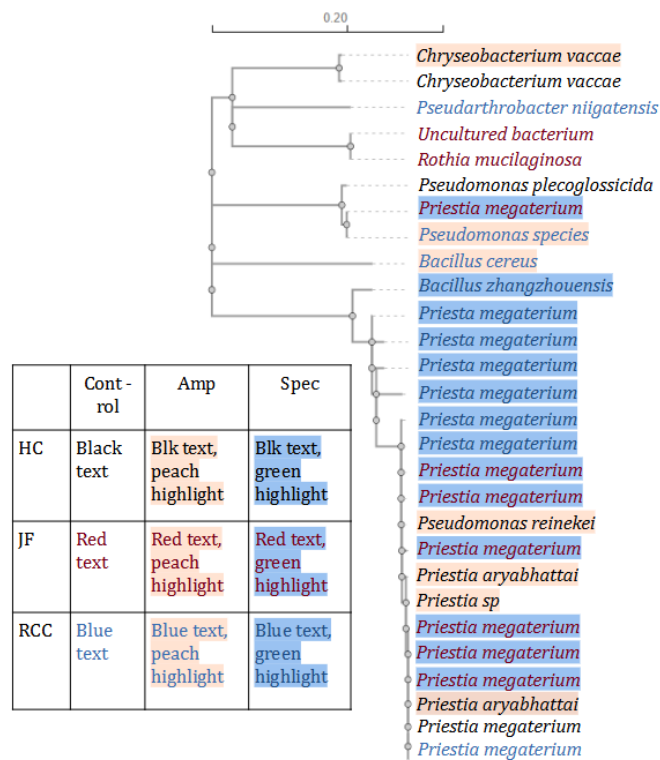


Figure 3. Dendrogram of bacterial isolates collected from soil samples. Genetic information was obtained using 16s RNA PCR and Sanger Sequencing. Legend depicts species' location and antibiotic resistance.

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