Background and Purpose:

Antibiotic resistance in bacteria is a constant threat to humanity, as new and improved antibiotics must be developed to combat the rise of new mutations leading to resistance. Humanity is in a constant arms race with the inevitability of antibiotic-resistant antibiotic bacteria, as several factors lead to the rise of new resistance tactics (Doron, 2008). Plasmids are circular strands of DNA found exclusively in Prokaryotic cells that specialize in the survivability of the cell, which includes the bacterium these strands inhabit. These plasmids also allow cell-to-cell communication of various bacterial cells, allowing resistance to be passed down quickly (Orlek, 2017). Resistance can also arise from mutations, and errors in DNA replication that may rarely be beneýcial to the host. Due to the rapid replication of bacteria, these mutations are much more likely to occur. Some of these mutations provide the bacterium with antibiotic resistance to prolong the survival of the cell (Watford, 2022).

These bacteria can occupy many surfaces, but ground soil is a common source of various forms of bacteria, including species unknown as of now (PARE, 2022). Exposure to bacteria of unknown origin can be potentially dangerous, as the degree of antibiotic resistance can be deadly when infections begin to arise. The goal of the PARE project is to document the accumulation of antibiotic resistance in a variety of mediums around the globe. These sites can be monitored to control the future spread of microbes, but a group e ort is required as many individuals can select speciýc locations to report ýndings (PARE, 2020). The objective for the following procedure will focus on the soil medium, and the site will be The Lake Forest College Health and Wellness Center. This site is important, as antibiotic resistance can arise in the soil through animal feces, thrown out prescription medications, sources of water including ýltration plants and rivers, and water runo from sources of agriculture (Doron, 2008). The usage of antibiotic prescriptions leads to an increase in antibiotic resistance, even if indirectly applied to an environmental source. A study has shown that poultry treated with antibiotics leads to an increase in Salmonella cases of the consumers of the meat (Levy, 1976). The feces of poultry also make their way into the soil, which would further spread the presence of antibiotic-resistant bacteria treated with the initial antibiotic.

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tance compared to other local sites. A set of serial soil dilutions will be performed to prepare ýve individual bacterial mediums, in which growth will be observed on speciýed petri dishes. As the concentration through dilutions decreases, it is predicted that growth will slow down exponentially. For petri dishes with antibiotic treatment, fewer colonies are to be expected. However, a mathematical approach to calculate the percent of colony-forming units will be used to determine the relative percent of antibiotic resistant bacteria.

Procedure:

Safety:

For the series of wet labs, gloves, a lab coat, a mask, and safety goggles were prioritized. Unknown bacteria were likely to be encountered throughout the preparation. Contact with materials was handled with care, where standard sanitizer was used to remove any possible contaminants from personal belongings before proceeding to further steps of the experimental protocol. Pipettes used were to be placed þat on a surface if a tip was attached. If doubtful, a tip was removed and replaced with a fresh one. Any solid and liquid solutions or mediums used were always covered, except when following the experimental procedure.

Soil Collection:

An open area was located at the Lake Forest College Health and Wellness Center. The collection was authorized before commencing, as the site was the private property of campus grounds. The soil was collected while wearing sterile gloves, and a metal spatula was used to transfer an appropriate sample size into a test tube. The sample was roughly the size of a golf ball. The test tube with the sample was sealed tightly before being stored in a plastic zip-lock bag. After, the location of collection was marked on the PARE Project website, where resistance data could be implemented before bacterial incubation.

Plate Set 2:

CFU calculations for plate sets 1 and 2. Values from rows 1, 2, and 3 are multiplied to obtain the total CFUs per gram of soil in row 4. Red values indicate counted colonies taken from table 1.

Table 3:
Plate Set 1:

Plate Set 2:

Number of TeT resistant CFUs and percent resistant CFUs calculated for plate set 1 and 2. Red values from table 1, blue values as percent-based calculations using number of TeT resistant CFUs divided by number of untreated CFUs before multiplying by 100. Untreated CFUs from table 2.

Table 4 accumulates the data of the entire class where other students conducted the same procedure, except at di erent sites. The Percentages are all di erent from each other for both TeT concentration groups. Some locations present TeT resistance values hig

Future research with the PARE project could compare a variety of residential sites with the environmental dispersion of antibiotics. Hixon Hall has been observed to be a critical site when it comes to the dispersion of antibiotics. Potentially analyzing the soil would provide context as to how dispersion is controlled and provide future management to sites that have a high percent of resistant bacteria. Besides soil, the controlled medium could be adjusted to water. Bacteria can occupy a variety of various growth mediums, where water is another potential site of accumulation. Collecting water from the runo site of a residential or commercial complex would provide context as in how antibiotic compounds are dumped through wastewater. Based on the collection of water runo , would it be possible that residential sites with poorer management of antibiotics have a higher environmental percent of antibiotic resistance than those that are well maintained? Or would the percent of resistant bacteria remain the same?