

## Abstract

Antibiotic resistant bacteria infiltrate drinking water, increase human exposure and spread into clinical settings. Regulatory agencies need simple test for ARI to monitor environmental quality. This study is first to measure resistance in a consortium of bacteria, not pure cultures. Our rapid method – “In-the-zone” (ITZ) employs a) the size of Zones of inhibition b) Identity of bacteria in the zone c) number of resistant clones within zones of inhibition and d) type of antibiotic to compute ARI. Results can be obtained within 24 - 48 hrs with very little effort. This Semester, we repeated the test and validated the weighting of various parameters. The ITZ assay yield consistent and reproducible data on zones of inhibition of bacteria community. The diameter of inhibition zones was most important parameter distinguishing resistomes of varying ARI. Landfill, manured garden and waste plant samples scored highest in antibiotic resistance index compared to beach sand.

## Introduction

- Soil environments are the ultimate reservoir of the antibiotic resistant organisms
- Antibiotic resistance index (ARI) was developed by scientists in the past. This tedious and expensive process does not permit standardization of ARI since the diversity of bacteria varies in susceptibility making it difficult to confirm the isolates of the resistomes representative of the microbiome
- This calls for a simple test for ARI which could be used to monitor different environmental quality

## Objectives

- To obtain a parameter that could easily give as relative antimicrobial resistance of a community of bacteria in a niche. It is hypothesized that the zone of inhibition, minimum inhibitory concentration and the number of resistant clones within broad spectrum will be a good indicator. Adequate broad spectrum antibiotic would yield antibiotic resistance in a given assay

## Materials & Methods

### Materials

- Mueller Hinton agar and broth, and phosphate buffered saline
- Tetracycline 30 disk, Vancomycin 30 disk, Ciprofloxacin 5 disk, Clarithromycin 15 disk
- Petri dishes
- Thermocycling qiagen DNA kit

## Methods

- 1 Bacteria from 4 different beach sand, 2 garden soils, 1 landfills, 1 waste water treatment plant and 1 garden soil were extracted in a buffer, pelleted by spinning and normalized in a buffer
- 2 Equal volumes of PBS suspension of bacteria were used to create lawns on Mueller- Hinton agar plates before placing four standard antibiotic disks aseptically
- 3 Bacteria In-the-zone were point inoculated on sterile MHA plates to create a library of 260 organisms
- 4 Using microscopy and biochemical tests, the library was grouped into 26 operational taxonomic units (OTUs)
- 5 Genomic DNA of representatives were extracted using Qiagen tissue kit from overnight LB cultures
- 6 After amplifying the 16S gene by PCR, the samples are being sequenced for BLAST analysis using the RDP II database

## Results

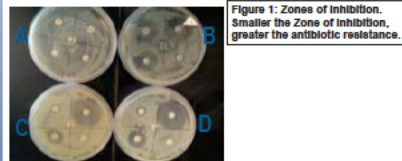


Figure 1: Zones of inhibition. Smaller the Zone of inhibition, greater the antibiotic resistance.

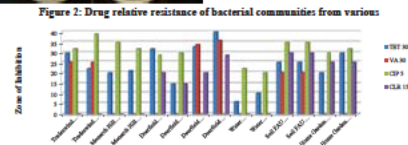


Figure 2: Drug relative resistance of bacterial communities from various

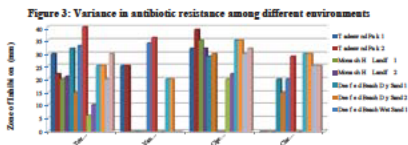


Figure 3: Variance in antibiotic resistance among different environments

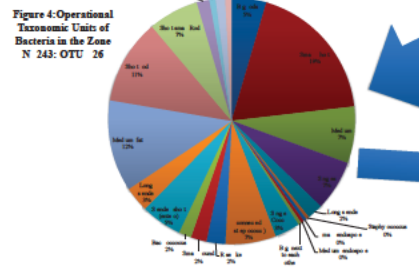


Figure 4: Operational Taxonomic Units of Bacteria in the Zone N: 243- OTU: 26

Figure 5:

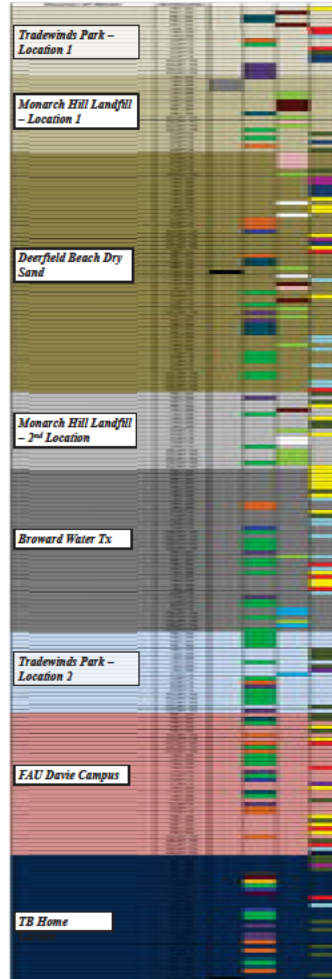
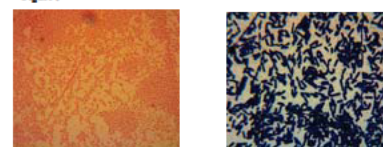


Figure 6:



## Results

- 1 Figure (2) Zones of inhibition ranged from 5-35 mm for all environmental bacterial community tested
- 2 Figure (3) indicates that hotspots for antibiotic resistant bacteria is park highly occupied by both people and hoses and landfill. This is based on small zones of inhibition meaning higher resistance to antibiotics
- 3 Based on zones of inhibition, it is interesting to note that water treatment plants was the least resistant based on zones of inhibition
- 4 Relative resistance of bacteria towards antibiotics vary greatly from each part of the consortium of samples using the –In-the-zone parameters
- 5 According to Figure (3) broad spectrum antibiotic Ciprofloxacin appears to be the most effective against environmental bacteria since it had the widest zone of inhibition
- 6 Frequency and prevalence of resistant bacteria in different environments can be seen in Figure (5)

## Discussion

- The ITZ assay yield consistent and reproducible data on zones of inhibition
- The diameter of the inhibition zones was the most important parameter and could clearly distinguish some resistomes of varying ARI

## Conclusion

We can use disk plate method of antibiotic assay to measure the resistomes and evaluate differences in resistomes of various environments as shown in Figure

## References

1. Esiobu N, Armenta, L and Ike JO (2002) Antibiotic resistance in soil and water environments used for various activities. *Int J of Environ And Health Research* 12 (2):133 –144
2. Marti, Elisabet, Juan Jofre, and Jose Luis Balcazar "Prevalence of Antibiotic Resistance Genes and Bacterial Community Composition in a River Influenced by a Wastewater Treatment Plant" *Ed Dwayne Elias PLoS ONE* 8 10 (2013): E78906
3. Kummerer, K. "Significance of Antibiotics in the Environment" *Journal of Antimicrobial Chemotherapy* 52 1 (2003): 5-7