Antibiotic Resistant in Commercial Septic Discharge

Jacob Schafer, Jay Nielsen, Michael Servi and Matt Haak

Faculty Sponsors: Sasha Showsh, Crispin Pierce, and Eli Gottfried

Introduction

- Antibiotic resistance is evident when a drug can no longer inhibit the growth of the target bacteria.
- According to the National Nosocomial Infections Surveillance (NNIS) System data on intensive care units (ICUs) in the U.S, 28.5% of enterococci associated with nosocomial infections in ICU patients in 2003 were resistant to vancomycin and resistance rates of *Pseudomonas aeruginosa* were 31.9% (to ceftazidime)
- More than 80 pharmaceuticals and drug metabolites, have been measured in µg/l-levels in sewage samples and downstream surface waters. (4)
- Recent research has shown certain bacteria may survive on a diet of the antibiotic Vancomycin. (5)

Materials and methods

1) **Twenty-Seven soil samples (19 control and 8 treated) were collected from a crop field near Eau Claire treated with septic system effluent (Figs. 1 and 2). These samples were mixed with water and plated on Colombian Blood Agar and five antibiotics (Kanamycin, Eosine Methylene Blue, Tetracycline, Erythromycin and Ampicillin, Fig. 3).**
2) **Bacterial antibiotic resistance generated from a large retail store and a small chain restaurant was measured. Several samples of effluent from the respective septic systems were analyzed for bacterial resistance to ampicillin, kanamycin, tetracycline, and erythromycin.**

Methods to conduct the experiment…

- Mix 10 grams of soil sample (or liquid effluent) in Erlenmeyer flask containing 50ml of water. Stir for 1 minute and let rest for another 1 minute
- Make three dilutions aseptically transferring 500µml of solution into a test tube
- Transfer 100µml of each diluted solution to a CBA plate or a CBA plate containing a particular antibiotic
- Store at 37°C for 24hrs
- Observe and count colonies.
- Use CFU/ml = Total Dilution Factor * 1/amount plated * number of colonies

Methods to collect the soil samples…

- Samples taken seven inches below surface from area near Eau Claire treated with septic effluent
- Surrounding non-treated area was used as control
- 27 samples collected and store on ice

Fig. 1 Land spreading of septic tank effluent.
http://www.limemaster.com/Land_Spreading.html

Fig.2 Soil Sampling Site

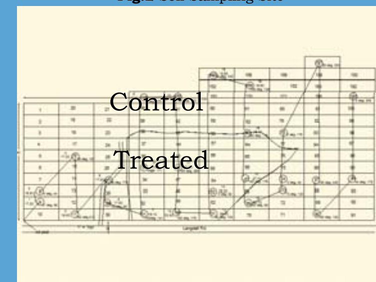


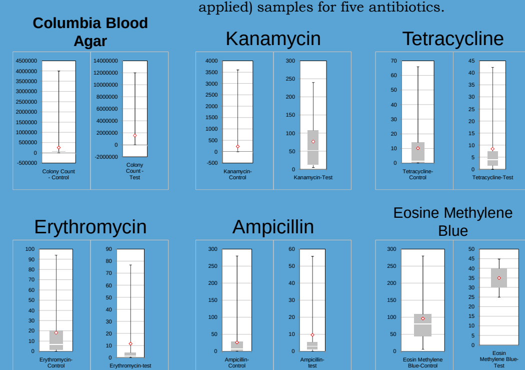
Fig. 3 Petri plates with sequential dilutions.
http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayarticle&art_id=273&theme=printer

Results

•Although the fraction of antibiotic-resistant colonies tended to be higher for control vs. treated samples, this apparent difference did not reach a significance of $p<0.05$ (one-tailed t-test, equal variance, Fig. 4).

•The restaurant contained higher levels of resistance to ampicillin, kanamycin, and tetracycline, with an average of 51.79%, 27.54%, and 30.78% of microbial growth resistant to the respective antibiotics. The store generated an average of 17.50%, 17.87%, and 5.97% resistance to ampicillin, kanamycin, and tetracycline. Erythromycin resistance was greater in effluent discharged from the store at 7.00% than it was in the restaurant at 2.87%.

Fig. 4 Colony growth and fraction of antibiotic resistance in control and treated (septic effluent-applied) samples for five antibiotics.



Conclusions

- **In this small study, treatment of an agricultural field with septic system effluent did not significantly change the fraction of bacteria that were resistant to five antibiotics (Kanamycin, Tetracycline, Eosine Methylene Blue, Erythromycin and Ampicillin).**
- **Measurements from the chain restaurant and large store are significant because they create a baseline percentage of normal or expected antibiotic resistance discharged from commercial sites.**

BIBLIOGRAPHY

1.Paterson, David . "Update on Antibiotic Resistance in Hospitals." The Prevalence of Antibiotic Resistance in the Hospital Setting. 2006. 20 Apr 2007 <http://www.medscape.com/editorial/cmetogo/5541>.

2. Fridkin, Scott K. et al. "Temporal Changes in Prevalence of Antimicrobial Resistance in 23 U.S. Hospitals." Emerging Infectious Diseases Vol. 8, No. 7,(2002): 697-701.

3. Lipsitch, Marc. "The epidemiology of antibiotic resistance in hospitals:Paradoxes and prescriptions." PNAS vol.97(2000): 1938-1943.

4. Herberer, Thomas. "Toxicology Letters: Occurrence, Fate, and Removal of Pharmaceutical Residues in the Aquatic Environment: a Review of Recent Research Data." ScienceDirect. 15 Mar. 2002. Toxicology Letters. 7 Apr. 2008

5. "Antibiotic-eating germ alarms doctors - Enterococcus faecium bacterium survives on diet of the antibiotic vancomycin - Biomedicine - Brief Article". Science News. Dec 21, 1996.