A Landscape Perspective on Antibiotic Resistance in Soil Bacteria

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Abstract Antibiotic resistance in bacteria is a pressing public health problem. Although most research on antibiotic resistance is focused on clinical settings, antibiotic resistant bacteria are also found in the environment. Selection for resistance in the environment may stem from the use of antibiotics in agricultural practices, from indirect selection for resistance to heavy metals, and from natural interactions among soil microbes. The extent, pattern, and causes of this environmental resistance are not well understood. We performed a novel study of the large-scale distribution of resistance in enteric bacteria inhabiting soil. We determined the relationship of antibiotic resistance in *Enterobacter aerogenes* to land use and concentration of heavy metals during July of 2007 and 2009 in Lancaster County, PA. Sites for soil collection were randomly chosen within each land use type. Measures of resistance involved the isolation and replica plating of 48 isolates per site. Growth at or above the threshold drug-level was deemed resistance. Metal analyses were performed with a MS-ICP. From soil samples collected from 84 sites in 2007, we found mean proportions of isolates resistant to ampicillin (0.50), chloramphenicol (0.49), kanamycin (0.05), and tetracycline (0.03). From soil samples collected from 73 sites in 2009, we found mean proportions of isolates resistant to ampicillin (0.64), chloramphenicol (0.12), kanamycin (0.002), and tetracycline (0.02). Levels of resistance were typically lower in forest versus residential, pasture, and crop land uses. In both years, bacteria isolated from forests had significantly less resistance to ampicillin (2007: $F_{3,80}$ = 6.7, p = 0.001; 2009: $F_{3,69}$ = 5.6, p = 0.002) than those in residential, crop, or pasture sites. In 2007, bacteria isolated from forests and residential sites had significantly less resistance to kanamycin ($F_{1,79}$ = 11.88, p = 0.001) than from agricultural sites (crop and pasture). We did not detect an effect of heavy metal concentration on antibiotic resistance. Our research documents current levels of antibiotic resistance in the environment and provides an initial assessment of variables affecting that resistance. We suggest a greater effort be made to understand the relationship between land use, soil characteristics, and antibiotic resistance in the environment and the implications of these relationships on public health.

Introduction

Widespread public concern about antibiotic resistance, highlighted by media attention on MRSA, masks the fact that relatively little is known about antibiotic resistance beyond clinical settings. In environmental settings, microbial resistance to antibiotics may evolve as a response to the administration of antibiotics to animals and crops, as consequence of non-anthropogenic microbial interactions, and as indirect effect of co-selection for resistance to heavy metal contamination. The extent, pattern, and causes of this environmental resistance are not well understood.

Study Aims

During July of 2007 and 2009 we conducted a novel, large-scale, systematic assessment in Lancaster County, PA of antibiotic resistance in the terrestrial environment to:

1) Quantify rates of resistances in *Enterobacter aerogenes*
2) Determine if resistance levels differ by land use (forest, crop, pasture, and residential)
3) Determine if resistance levels differ by soil metal concentration
4) Assess the relative role of anthropogenic land use and heavy metal concentration in influencing resistance

Methods

- Sampled July 2007 and July 2009
- All collection sites within Lancaster County, PA, USA
- Stratified Random Sampling Design by Land Use

Soil Sample

METAL ANALYSES

Soil digestion via EPA 3051 Microwave Digestion Method

Concentrations of trace elements measured by MS-ICP
ELEMENTS: Al, Ba, Fe, Co, Cd, Cr, Cu, V, Mn, Ni, P, Pb, and Zn

MICROBIAL ANALYSES

Enterobacter aerogenes	Antibiotic Resistance
Coliscan Easy Gel	Ampicillin (35 μg/ml)
EMB	Kanamycin (70 μg/ml)
Mannitol Salt Agar	Chloramphenicol (35 μg/ml)
	Tetracycline (20 μg/ml)

Results

- Data collected from 84 sites (2007) & 73 sites (2009), *Fig. 1*
  - Residential: 36 (2007); 17 (2009)
  - Forest: 18 (2007); 15 (2009)
  - Crop: 18 (2007); 23 (2009)
  - Pasture: 12 (2007); 18 (2009)
- High levels of resistance throughout the county for 2 of the antibiotics, *Fig. 2.*
- Ampicillin resistance was significantly lowest in forests, *Fig. 3.* Kanamycin resistance in 2007 was lowest in forest (p=0.009) and residential (p=0.04) sites. Resistance to other antibiotics did not statistically vary among land use.
- Multiple linear regressions of principle components of soil trace elements versus each antibiotic revealed a complex relationship, *Table 1*. We did not detect a relationship between a single heavy metal concentration and resistance, *Fig. 4*.

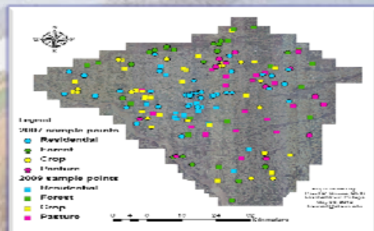

Figure 1: Sites sampled during 2007 (n=84) and 2009 (n= 73). Sites were determined using a GIS. A random design stratified by land use (residential, forest, crop, & pasture) was used.

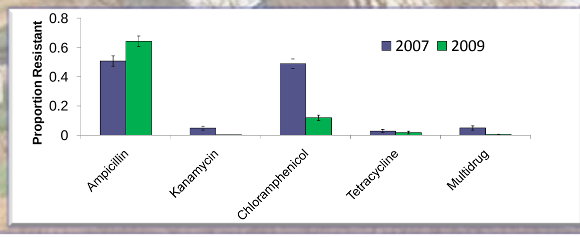

Figure 2: Proportion of *Enterobacter aerogenes* resistant to antibiotics (+1 Standard Error). Multidrug indicates resistance to 3 or more of the 4 antibiotics tested.

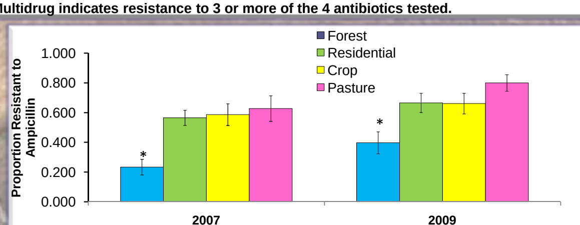

Figure 3: Proportion of *Enterobacter aerogenes* resistant to ampicillin (+1 Standard Error) by land use in 2007 and 2009. * 2007: $F_{3,80}$ = 6.7, p = 0.001; 2009: $F_{3,69}$ = 5.6, p = 0.002).

Table 1: Best model resulting from a backwards multiple linear regression of each individual antibiotic versus the five principle components. The principle components are: PC1 = Cr, Co, Ni, Cu, V; PC2 = Be, Mn, Ba; PC3 = Zn, Pb; PC4 = Al, Fe; PC5 = Cd, P.

Antibiotic	Principal Component	Best Model
Ampicillin	PC5	p = 0.003; r^2 = 0.062
Kanamycin	PC1, PC4	p < 0.001; r^2 = 0.143
Chloramphenicol	PC1, PC3, PC4, PC5	p < 0.001; r^2 = 0.271
Tetracycline	none	None significant
Multiple drug	PC1	p < 0.001; r^2 = 0.113

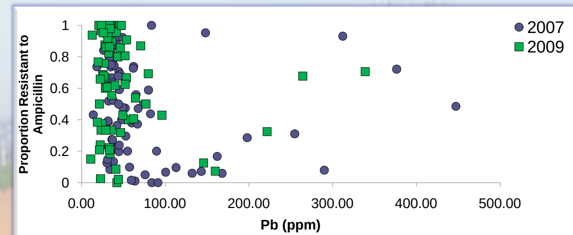

Figure 4: Proportion of *Enterobacter aerogenes* resistant to ampicillin by lead (ppm) in 2007 and 2009.

Discussion

Large-scale environmental sampling is a new approach to investigating antibiotic resistance. A greater understanding of resistance in the environment can help identify potential reservoirs for new antibiotics, movement of resistance from environmental samples to human pathogens, and help us devise better antibiotic use policies and surveillance.

Using a large-scale environmental sampling approach in July of 2007 and 2009 in Lancaster County, PA, USA, we document current levels of antibiotic resistance in the environment. We found:

- High levels of microbial resistance for 2 antibiotics (ampicillin and chloramphenicol)
- Land use partially explains patterns of resistance. When antibiotic resistance by land use was tested, forested sites had the lowest levels of resistance. No other antibiotic resistance patterns were observed.
- A combined effect of soil trace elements on antibiotic resistance was detected. No single heavy metal concentration on resistance was significant, although at the observed low levels of metal, there may not be a selective pressure.

A greater effort should be made to understand the relationship between land use, soil characteristics, and resistance and the implications of these relationships on public health.

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